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THOMPSON YATES LABORATORIES

REPORT

THE
THOMPSON YATES LABORATORIES
REPORT

EDITED BY

RUBERT BOYCE AND C. S. SHERRINGTON

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OF THE

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OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE
AND MEDICAL PARASITOLOGY

BY

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AND

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PART II. FILARIASIS

WITH ILLUSTRATIONS AND PLATES

AT THE UNIVERSITY PRESS OF LIVERPOOL 1901

ISSUED BY THE COMMITTEE

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

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PREFACE

The series of new blood filariae described in the following pages were found during the examination of a large number of West African birds of different species for parasites of the red blood corpuscles. The discovery of the blood filariae naturally led to a search for their parent forms: but time did not permit of any extensive investigations being made as to the nature of their intermediary hosts.

Opportunities also occurred for observations on human filariasis in West Africa, which combined with the work on avian filariasis, will, it is hoped, throw considerable light on this very interesting branch of parasitology.

The description of the parasites has involved a great amount of labour in the examination of the literature of the subject, and for this reason, and also because of the rapidly increasing importance of the subject, and of the desire for a comprehensive work, often expressed by investigators in tropical countries, it has been considered desirable to incorporate in this work STOSSICH's extensive bibliography, and also to introduce chapters on the Nematodes in general and the Filariae in particular, for the greater part of the matter of which we are indebted to the valuable works of SHIPLEY (*Worms*, etc., The Cambridge Natural History) and RAILLIET (*Zoologie médicale et agricole*).

The authors wish particularly to thank Mr. A. E. SHIPLEY for his useful advice and help; Mr. ROBINSON, who kindly undertook the identification of the birds of our collection; Dr. A. H. HANLEY for much valuable material; and our colleagues at University College for their assistance.

H. E. A.
J. E. D.
J. H. E.

September, 1901

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REPORT OF LIVERPOOL EXPEDITION TO NIGERIA

PART II

I. *FILARIASIS*

INTRODUCTION

THE NEMATHELMINTHES, the order to which the genus *Filaria* belongs, have the following characteristics :—they are worm-like in form, but non-segmented; that is, their bodies are not divided into segments, each resembling more or less exactly in outward appearance and internal structure the preceding and following segment. Many bear bristles or hooks, and exceptionally suckers. The body is elongated, thread-like, enclosed in a more or less thick cuticle. They have no closed vascular system nor special respiratory organs. They are almost all dioecious—the male and female reproductive organs being in different individuals. The young somewhat resemble the adults, but have no sexual organs ; the immature stages, termed *larvae*, are often free while the adults are parasitic or *vice versâ*, or inhabit a different host from the adult. Some of these Nemathelminthes spend their life within the bodies of their hosts, or are only parasitic during a portion ; a few have a free life in water or damp earth.

The Nemathelminthes comprise three sub-orders :—

1.—The Nematoda
2.—The Nematomorpha (Gordiidae)
3.—The Acanthocephala

The Nematoda have a complete digestive tube ; in the Nematomorpha it is atrophied in the adult, while in the adults of Acanthocephala it is absent altogether. In the sub-order Nematomorpha (Gordiidae) are two genera, *Gordius* and *Nectonema ;* the latter has only a single species, *Nectonema agile*, which is marine. The genus *Gordius*, which is entirely fresh water, has a large number of species. Worms of this genus pass through three stages, two larval and parasitic, the third, sexually mature, living in water. The first larval stage has been found in the larvae of *Sialis lutaria Ephemera*, *Tanypus*, *Corethra*, *Chironomus* ; the second is parasitic in the bodies of *Carabus hortensis*, *Procerus* (*Carabus*), *Coriaceus*, *Calathus fuscipes*, *Molops elatus*, several species of *Pterostichus*, and other beetles.

According to RAILLIET[1] cases have been recorded in which the adult forms of some species of this genus have been evacuated after the administration of an anthelmintic—in some of the cases troublesome symptoms occurred. The actual

1. Railliet, *Traité de Zoologie Medicale et Agricole*. Paris, 1895. P. 563.

species described as occurring thus, are :—*Gordius aquaticus*, *G. tolosanus*, *G. varius*, *G. chilensis*. Probably they gain access to the alimentary canal of man and animals through the medium of drinking water.

The Acanthacephala include, following SHIPLEY,[2] four families:—Neorhynchidae, Gigantorhynchidae, Echinorhynchidae, and Arhynchidae. The adult forms have no alimentary tract, and are provided with a retractable proboscis, armed with hooklets, arranged in longitudinal rows. The adult stage occurs in the alimentary canal of vertebrates, generally those which live in or near water; while the larvae are found in the bodies of certain invertebrates, generally small Crustacea[3], *e.g.*, *Gigantorhynchus gigas* inhabits the small intestine of the pig, wild boar, and occasionally man, while the larval host is believed to be some species of beetle (*Melolontha*, *Cetonia*, and *Lachnosterna*). *G. echinodiscus* inhabits the intestine of ant eaters; *G. spira* of the king vulture; *Echinorhynchus proteus* of fishes (gudgeon, trout, turbot, etc.); the larval stage in some Amphipod Crustacea, and some fresh water fishes (minnow, etc.). Other species of Echinorhynchus occur in the duck, dog, rabbit, some aquatic birds, and occasionally man.

The *Nematodes* present very great difficulties to the systematist in their classification. SCHNEIDER[4] divided them into three groups:—(i) the Polymyarii, in which numerous muscle cells are seen in a transverse section; (ii) the Meromyarii, in which only eight are seen, two in each quadrant; (iii) the Holomyarii, in which the muscles are either not divided or only divided by longitudinal lines. Other classifications have been based upon the life history, but in many cases this is only very imperfectly known. At present the arrangement of the muscles (Polymyarii, Meromyarii, Holomyarii), the arrangement of the lips and mouth parts, the character of the male tail, the number of papillae, and the number and size of spicules, are the features which are relied upon for classification. SHIPLEY[5] deems it advisable at present to abandon the larger groups, and to deal directly with families. Of these he quotes seven:—

I. Ascaridae.
II. Strongylidae.
III. Trichotrachelidae.
IV. Filariidae.
V. Mermithidae.
VI. Anguillulidiae.
VII. Enoplidae.

We have considered it advisable to state briefly here the characteristic features of each of these families, and to describe shortly those forms of each family which are interesting to the student of human parasitology.

2. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms, Rotifers, and Polyzoa*. London, 1896. P. 182
3. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms, Rotifers, and Polyzoa*. London, 1896. P. 174.
4. Schneider, *Monographie der Nematoden*. Berlin, 1866.
5. Harmer and Shipley, *The Cambridge Natural History*, Vol. II, *Worms*, etc. P. 138.

I. Ascaridae

Shipley[6] gives the following characteristics: 'Body rather stout. A dorsal and two ventro-lateral lips bearing papillae. Buccal cavity distinct, seldom provided with chitinous armature. The oesophagus has two dilatations. The tail of the male is ventrally curved, and usually there are two horny spicules.' The females have a double ovary, and are generally oviparous.

Genera: *Ascaris*, *Heterakis*, *Oxyuris*, *Nematoxys*, *Oxysoma*, and others.

Genus *Ascaris*. These are polymyarian and have three lips, generally bearing teeth. The males have two equal spicules and a number of pre- and post-anal papillae, by the latter of which the best specific characters are furnished. The vulva is situated about the middle of the body. The ova are globular or ellipsoidal. They inhabit the intestines of their respective hosts. The species are very numerous.

The *life history* has not been completely worked out. Infection experiments by feeding directly with material containing ova have always failed. It is probable that the larval stage is passed in some intermediary host, and Von Linstow has lately suggested the millipede (*Julus guttulatus*) in the case of *Ascaris lumbricoides*.

Genus *Heterakis*. Also polymyarian, distinguished from the Ascarides by the presence of a ventral sucker and two often unequal spicules in the male. The male tail has also two series of papillae symmetrically placed, and often two lateral cuticular expansions representing a bursa. Almost all are oviparous. They live in the intestines of vertebrates, particularly of birds. There are several species, found in the fowl, turkey, duck, pigeon, pheasant, bustard, peacock, etc.

The *life history* seems to be simple, at least in the case of *H. taché*, the embryo developes from the ovum in moist media in about seventeen days, and when these ova containing embryos are given to pigeons, adult *Heterakis* are produced in three weeks.

Genus *Oxyuris*. Meromyarian, have three slightly-projecting lips. Oesophagus long with distinct bulb. Males are small and scarce, have a single spicule; two pairs of pre-anal papillae. Females have a long capillary tail, two ovaries, vulva opens in anterior portion of body. Ova are oblong and symmetrical, and often contain an embryo before parturition. Many species inhabit the intestines of man, horse, hare, rabbit, and iguana; and others the rectum of insects, cockroach, water beetle, etc.

The *life history* is simple—the ova, containing developed embryos are taken directly into the alimentary tract, and develop into adult worms.

Genus *Nematoxys*. Meromyarian has very complete arrangement of muscles and forms a transition to the polymyarian type.[7] The whole body of both sexes is

6. Harmer and Shipley, *The Cambridge Natural History*. Vol. II. P. 138.
7. Harmer and Shipley, *The Cambridge Natural History*. Vol. II. P. 142.

covered with numerous irregularly scattered papillae. There are but few species—found in snakes, amphibia, and eels.

Genus *Oxysoma* has but three species—found in the intestines of opossums, frogs, and turtles.

II. Strongylidae

Long cylindrical body, seldom filiform or capillary. Mouth surrounded with papillae, probably always six in number; often has an armature of teeth or spines. No distinct oesophageal bulb. The male orifice at the tail end is surrounded by a bell-shaped bursa, with one or two spicules. The female has one or two ovaries: the vulva is sometimes anterior, sometimes posterior to the middle of the body, sometimes near the anus. Ova are already segmented or contain embryos on leaving vagina.

Genera: *Eustrongylus*, *Strongylus*, *Dochmius*, *Sclerostomum*, *Cucullanus*, *Syngamus*, *Pseudalius*, *Ollulanus*, *Oesophagostoma*, and others.

Genus *Eustrongylus*. Cylindrical. Mouth has no lips, but is surrounded by papillae. Male has a filiform spicule; female a single ovary, vulva in anterior part of body.

Only two species known: *E. Gigas*, which inhabits the kidney capsules of carnivorous animals, especially of those which eat fish—dogs, seals, etc., and occasionally man, horse, and deer; and *E. tubifex*, found in aquatic birds—ducks, grebes, divers, etc.

Life history: In case of *E. gigas* the eggs are eaten by fish, the larval stage being passed in the peritoneal cavity of some fishes.

Genus *Strongylus*. Body slender; anterior end sometimes winged. Mouth often indistinctly lipped, has six small papillae. Males have a conspicuous genital bursa, strengthened by variously arranged ridges, which are of specific value. Female posterior end pointed, vulva almost always in posterior half of body.

There are numerous species found in mammals, birds, and reptiles. Some inhabit the intestine; others form aneurisms in the large blood vessels, particularly of horses; others live in the tracheae and lungs of sheep and cattle. They have been found in respiratory tract of the sheep, goat, ox, calf, pig, horse, cat, rabbit, hare, deer, buck, gazelle, ass, dromedary, etc.; in alimentary tract of sheep, goat, chamois, ox, deer, pig, horse, rabbit, etc.; in circulatory system of dog and horse.

Life history: (1) Those of the digestive tract have a rhabditiform embryo[8] provided with an oesophageal bulb, with three chitinous teeth. This embryo lives and grows on the organic matter in mud, and undergo a direct development. (2) Those of the respiratory tract produce larvae with an indistinct oesophageal bulb with no teeth; they do not grow in mud. Their development has not been followed, possibly they have an intermediary host.

8. Raillict, *Traité de Zoologie Medicale et Agricole*. Paris, 1895. P. 424.

Genus *Dochmius*. Anterior end turned towards dorsum. Mouth oval, limited by a chitinous border, followed by a chitinous buccal capsule, the dorsal wall of which is shorter than the ventral, and is supported by a conical rib, the point of which may project into the cavity. At the bottom of the capsule on the ventral wall are two teeth; towards the free edge the ventral wall also bears two other teeth, which are hooked at their extremities. The dorsal free edge is also sometimes similarly toothed. There are several species inhabiting the intestinal canal of man (*D. or Ankylostoma duodenale*), anthropoid apes, dogs, cats, sheep, and goats, wolf, fox, etc.

Life history: According to RAILLIET[9] and others, the embryos which hatch out, from the already segmented ova a few hours after leaving the intestine, under favourable conditions and after several moults, reach a stage in which they again, on gaining access to the alimentary canal, develop into adult ankylostomes. He mentions that LEICHTENSTERN has asserted that some larvae become transformed into sexually mature rhabditiform adults, which again produce larvae. GILES[10] also reports having traced the life history of the parasite through a sexually mature rhabditiform stage, the larvae of which become adult ankylostomes in the intestine of man.*

Genus *Sclerostomum*. Truncate anterior extremity, straight or slightly curved towards the ventral surface. Mouth circular, open, followed by a chitinous buccal cavity furnished along its edges with numerous teeth, disposed in one or several series. Male has two spicules and a generally tri-lobed caudal bursa. Vulva of female opens in posterior part of the body.

Several species have been found in the intestinal canal of the horse, mule, sheep, goat, deer, roe, antelope, etc.

Life history: RAILLIET[11] describes the following in the case of *S. equinum:* the eggs, passed with faeces, develop in water into embryos, which are taken up again probably in drinking water. They probably pass from the intestine into the circulatory system, and after a sojourn there return to the mucous membrane of the caecum, where they remain until a definite stage is reached, whereupon they pass into the intestine and pair. GILES,[12] however, in the case of *S. tetracanthum*, says that rhabditiform adults are produced as in the case of *Dochmius duodenale*.

Genus *Cucullanus*. Exists in the adult form in the intestines of fishes and reptiles. One species (*C. elegans*) lives in fresh water fish, *e.g.*, perch; while the young inhabit the body cavity of the crustacean *Cyclops*.

Genus *Syngamus*. Head end thickened. Mouth large. Chitinous buccal capsule. Males small; two spicules. Females have double ovary; vulva situated in anterior part of body; the male is generally permanently attached to the female, its genital bursa being closely adherent to the vaginal opening.

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9. Railliet *Traité de Zoologie Medicale et Agricole*. Paris, 1895. P. 467.

10. Giles *Report on Kala-azar and Beri-beri*. Shillong, 1890.

* The recent researches of one of us (Annett) tend to confirm the truth of these investigations.

11. Railliet, *Traité de Zoologie Medicale et Agricole*. Paris, 1895. P. 459.

12. Giles, *Some observations on the Life History of Sclerostomum tetracanthum : Scientific Memoirs by Medical Officers of the Army of India*. Part VII. Calcutta, 1892.

The parasites inhabit the tracheae and bronchi of birds and mammals, fowl, pheasant, turkey, peacock, partridge, magpie, crow, duck, goose, etc.

Life History: The ova escape from the body with fully formed embryos in them, by the decay or rupture of the parent. They hatch in damp earth or water in from one to six weeks, and on being swallowed develop into adults which produce eggs in less than three weeks.

Genus *Ollulanus.* The name is derived from the characteristic appearance of the chitinous buccal capsule, which is urn shaped. The male has two short spicules; the female a single ovary.

One species only is known, *O. tricuspis*, found in the intestine, bronchi, and other parts of the cat. The larvae become encysted in the muscles of the mouse.

Genus *Oesophagostoma.* Small circular mouth has a chitinous ring around which the cuticle is raised into a transparent pad on which are six sharp papillae. The pad is separated from the body by a constriction behind which the integument forms an ovoid swelling well limited posteriorly, at the level of a transverse cleft which occupies the whole breadth of the inferior surface. A few species are known which inhabit the intestine of the ox, horse, chamois, sheep, etc.

Life History: The adults are free in the intestine, the larvae live in small tumours in the mucous membrane.

III. Trichotrachelidae

This family is characterised by the anterior end of the body being long and whiplike, the posterior somewhat swollen. The mouth has no papillae; there is no oesophageal bulb. Males may have no spicule, or more often a single spicule surrounded by a sheath. The females have a single ovary; the vulva is situated at the beginning of the thicker portion. Some are ovoviviparous, others oviparous. Their eggs have two characteristic poles.

Genera: *Trichocephalus*, *Trichosoma*, *Trichina*, and others.

Genus: *Trichocephalus.* The anterior and posterior parts well marked. The ventral surface shews anterior by a broad longitudinal band formed by a number of punctiform projections. The male tail is twisted spirally, with its concavity corresponding to the dorsal surface. The female has a single ovary.

Several species are known, inhabiting the intestine of man (*T. dispar*), monkeys, lemurs, swine, hog, peccary, dog, cat, sheep, deer, ox, etc.

The *life history* is simple; there is no intermediary host.

Genus: *Trichosoma.* The posterior part containing the intestine and generative organs, is but very little swollen. The posterior end of the male has no papillae, but bears a rudiment of a bursa.

Parasites of birds and mammals. In mammals, different species live in the bladder of the fox and wolf, and of the cat, and in the trachea of the fox and martin. In some species two, three, or four males live within the uterus of the female.

Genus *Trichina*. Small capillary worm, slightly swollen posteriorly. Male has two conical appendages posteriorly forming a sort of copulatory bursa. There is no spicule. Female is viviparous. Vulva situated in anterior fifth of the body.

A single species *T. spiralis* only known.

Life history. The adults, male and female, live in the intestine of man and other mammals. The female produces very numerous eggs which give rise to embryos in the body of the uterus. These embryos bore through the intestinal wall of their hosts, and make their way all over the body, coming to rest most usually in the muscles. Here they generally pierce the sarcolemma and become encysted inside the muscle fibre. The larvae may here remain dormant for many years, and undergo fatty or calcareous degeneration. When trichinised meat is eaten, unless thoroughly cooked, the cysts are dissolved and larvae set free, and become sexually mature in three or four days ; again producing ova and embryos which bore through the intestinal wall.

IV. Filariidae

Long filiform worms ; mouth with two lips or without lips—often have papillae, and sometimes a buccal capsule. Males have a tail generally incurved, have one or two unequal spicules, four pairs of pre-anal papillae, and sometimes an unpaired one as well. Females have double ovary. Vulva is situated towards the anterior part of the body. Many are ovoviviparous.

Genera : *Filaria*, *Ichthyonema*, *Hystrichus*, *Spiroptera*, *Disparagus*, and others.

Genus *Filaria*. See next chapter.

Genus *Ichthyonema* is confined to fishes. Male is very minute, and the female partly degenerate. No anus, no external generative organs. Uterus fills the entire body cavity.

Genus *Hystrichis*. The anterior part of the body is armed with spines. Male has a bell-shaped bursa, and very long spicule. Vulva is near the anus.

The parasite lives between the walls of the oesophagus and gizzard of some birds—palmipeds (duck, swan).

Genus *Spiroptera*. These can only be distinguished from the *Filariae* by two features—the body is generally shorter and thicker, and the vulva is ordinarily nearer the mouth. Their specific name is taken from the tail of the males, which is rolled into a spiral and furnished with lateral membranous expansions.

Several species are described generally met with in tumours of the oesophagus, stomach and intestines of horses, asses, mules, pigs, dog, wolf, etc. *S. reticulata* has been found in the cervical ligament, in periarterial tissue, between muscles and tendons, and in other positions in the horse.

Life history is unknown—an insect is supposed to act as an intermediary host.

Genus *Dispharagus*. These have the oesophagus divided into an anterior straight tubular portion, and a long thick muscular posterior portion with a bulb. Male tail extremity is more or less coiled, and has lateral expansions : four pre-anal papillae on each side, two unequal spicules. Female has a simple ovary, and is oviparous. The several species occur in the oesophagus and stomach of some birds.

V. Mermithidae

Mouth has six papillae. There is no anus. Males have two spicules and three rows of numerous papillae. Body of female reduced to a simple sac, crowded with ova.

Genera : *Mermis*, *Bradynema*, *Atractonema*, *Allantonema*, *Sphaerularia*, etc.

These are parasitic in some stage on insects, *e.g.*, the sexually mature forms of genus *Mermis* live in damp earth, while the larval stage find their way into grass-hoppers, caterpillars, etc. The adult stage of *Bradynema* live in the body of small beetles, then reach the intestine, and eventually earth, where the females die, and the males, having developed spermatozoa in the larvae stage, now develop ova (protandrous hermaphroditism). The *Allantonema* have a somewhat similar history, as have also, *Atractonema* and *Sphaerularia*. The two last have the peculiar feature that at the time of sexual maturity a swelling—a prolapsus of the uterus and vagina—develops posteriorly and grows until it far exceeds the size of the worm. *Sphaerularia* are parasitic in the body cavity of many bees (Bombyx).

VI. Anguillulidae

These are for the most part free living and small. Oesophagus has two bulbs, the posterior without teeth. Buccal cavity contains a small spine. Males have sometimes a bursa with no papillae ; two equal spiculae. Females have a double ovary, and vulva in posterior half of body ; often ovoviviparous, but the number of embryos is small.

Genera : *Diplogaster*, *Mononchus*, *Rhabditis*, *Tylenchus*, *Anguillula*, *Heterodera*, etc.

Many species of this family live in humus or decaying matter, others are parasitic in plants ; some live in organic matter, and some few are parasitic in animals.

Tylenchus, *Aphelenchus*, and *Heterodera* infect plants and give rise to 'sickness' among clover, rye, oats, onions, beet, etc.

Genus *Rhabditis*. Oesophagus has two bulbs, posterior, and sometimes with teeth. Buccal cavity no teeth nor spines. Males may have a caudal bursa ; often has six to ten papillae on bursa or in middle line, two short spicules with an accessory piece. Some species are hermaphrodite.

Some species live in moist earth, others are described as causing a disease resembling typhoid, and larvae of species have been formed in the papules of some skin eruptions in man and dogs.

Genus *Anguillula*. Oesophagus has two bulbs, posterior has no teeth. Male is provided with a bursa with no papillae. The uterus is asymmetrical.

Numerous species are parasitic on plants, wheat, etc. *Anguillula aceti* is found in vinegar and paste. Others present two mature generations which succeed each other, (1) a free form, dioecious, resembling Rhabditis, and (2) a hermaphroditic form which is parasitic.

OERLEY places these in a new family, *Angiostomides* with three genera—*Angiostoma*, *Strongyloides* and *Allantonema*.

Anguillula intestinale (Strongyloides intestinale, Anguillula stercoralis) is parasitic in the intestine of man, giving rise to some forms of diarrhoea and dysentery (Cochin China), and produces ova which give rise to rhabditiform larvae, which are passed with the faeces. In the soil these become sexually mature, pair and produce larvae, which eventually reach the digestive tube to become *Anguillulae intestinale*.

VII. ENOPHIDAE

These are free living, small, usually marine; devoid of a second oesophageal bulb. Eyes and mouth armature often present. Fine hairs and bristles surround the mouth.

Genera: *Enoplus*, *Dorylaimus*, *Enchelidium*, etc. Some species are parasitic on plants, others on the sea urchin, and other animals.

II. THE *FILARIAE*

The genus *Filaria* is a very large one. It appears to be confined to vertebrates, usually living in the tissues of the body and not in the intestines. The worms are remarkable for their long slender bodies, which are almost of the same breadth throughout the whole length. The anterior extremity is rounded, and often has no lips. The males, which are markedly smaller than the females, have an incurved or spiral tail sometimes furnished with lateral expansions ; more often they possess four pre-anal, and a variable number of post-anal papillae ; the spicules vary considerably in size and appearance. In the females the vulva opens more or less near the mouth.

Filaria which are Parasites of Man

Filaria bancroftii *Filaria diurna* *Filaria perstans* *Filaria ozzardi* *Filaria magalhesi* *Filaria demarquaii* *Filaria loa*	These will be described in a subsequent chapter.

Filaria medinensis

Guinea worm : The adult female is a white or yellowish worm, averaging about sixty centimetres long, though specimens reaching four metres in length have been described. Its breadth, which is uniform, is from 0·5 to 1·7 mm. The anterior extremity, which tapers slightly, is truncated, and presents a rugous cuticular thickening in the centre of which is the triangular buccal orifice. The thickening bears two large papillae, one dorsal and one ventral, and six small papillae. The body shows faint transverse striation. The cuticle is thick. The musculature is polymyarian. The tail incurved towards the ventral surface in the matured females, terminates in a sharply bent hook about 1 mm. in length. The alimentary canal consists of a fine tube running from the mouth to near the tail, but not opening externally in the gravid female, though an anal orifice exists in the young parasite. In the mature worm the uterus crowded with embryos fills the whole body cavity—vulval opening and vagina being obliterated. The embryos, usually lying curved on themselves *in utero*, measure 15 to 25 μ long by 0·50–0·70 μ wide. They are slightly flattened, transversely striated, and provided with a finely tapering tail which measures about two-fifths of their whole length. They have a rudiment of an

alimentary canal, and bear two small lateral sack-like structures at the base of the tail. They swim actively and may live for days in muddy water and damp soil. They are said by some authors to escape only by rupture of the adult worm, but according to MANSON[1] they are emitted by a prolapsus of the uterus through the mouth. The mature worm drills a hole in the derma. Over this the epidermis forms a bulla, which ruptures in a few days, disclosing a small superficial ulcer with a hole at its centre, under which lies the head of the worm. On the application of water to the ulcer, a minute quantity of whitish fluid is extruded, seen on microscopical examination to be swarming with embryos; or a little tube, the prolapsed uterus itself, is sometimes seen protruding. In about a fortnight the whole uterine contents are emptied. It is usually asserted that the female alone is known, and that it is uncertain whether it is hermaphrodite or whether both sexes are present in the *Cyclops*. CHARLES[2] has described a specimen found in the mesentery of a human subject from an orifice in the middle of the body of which he drew out a much smaller specimen, which may have been the male.

Life history. The young embryos in water attack a fresh-water *Cyclops* and penetrate through the interarticular membrane between the abdominal plates into the body cavity. Here the intestine of the parasite further develops, and on the eleventh day they moult and exhibit a very changed appearance, being shorter (0·5 mm.) and non-striated. In four weeks they measure 1 mm. in length. They are thought to reach man again through the medium of drinking water containing infected *Cyclops*: the parasite being able to pierce the tissues to reach its usual site in the legs. CHAPOTIN and others claim that the embryos can enter the body through the skin. PLEHN[3] reports to have fed two monkeys on bananas covered with embryos, and that one of them subsequently developed a painful tumour of the thigh and died after eight and a half months. The tumour contained a worm in all respects identical with *F. medinensis*, though only 4·0 cm. long.

Filaria lentis. Diesing

Syn. *F. oculi humani*, VON NORDMANN. Under this title are included nematodes, found on several occasions in the eye of man. Those described have varied considerably in length, 1·72 to 12·6 mm. RAILLIET[4] considers that they represent worms of different species which have gained access to the wrong host, or such as have been arrested in their development. Specimens have been described by VON NORDMANN, GESCHEIDT, and SCHÖLER.

Filaria inermis. Grassi

Syn. *F. palpebralis*, PACE, nec WILSON; *F. peritonei hominus*, BATES; *F. conjunctivae*, ADDARIO. The female only is known. It measures about 160 mm. long

1. Manson, *Tropical Diseases*. 1900 p. 554.
2. Charles, a Contribution on the Life History of the male *Filaria Medinensis*, founded on the examination of specimens removed from the abdominal cavity of man. *Scientific Memoirs, by Medical Officers of the Army of India.* Part vii. Calcutta, 1898.
3. Plehn, *Die Kameru-Kuste*, etc. Berlin, 1898. p. 295
4. Railliet, *Traité de Zoologie Medicale et Agricole*. Paris, 1895. p. 529.

by 0·475 mm. broad. It is of whitish or brownish colour, somewhat flattened, threadlike, and tapers slightly towards both extremities, but more especially posteriorly. The extremity of the tail is incurved. Cuticle is transversely and longitudinally striated, except at the cephalic end; musculature polymyarian. The mouth is very small, unarmed, and terminal; oesophagus is short (620 μ), widens somewhat at hinder end. The anal aperture is 300 μ from the tip of the tail, the vulva 50 to 104 μ from the mouth. The eggs hatch out in the uterus; the free embryos measure 350 μ by 5·5 μ, and taper slightly in front, sharply pointed posteriorly. A peculiar formation, probably of glandular nature, occurs at the point of the tail where the cuticle is thin: on each side of the thin portion is a break, with a corresponding canal which resembles the duct of a gland. In some examples there is a third one between the other two, but its outer opening was not made out.

Life history unknown. The adults have been found in man, the horse, and donkey. In man they have been found three times in the eye: once encysted in the gastro-splenic omentum, and once encysted in the ocular conjunctiva. *Filaria lentis* (DIESING) may be a young form of this worm.

Filaria volvulus. Leuchart

The description of the male and female of this worm is given by PROUT.[1] The female is 40·4 cm. long and the body 0·34 mm. wide, which gradually tapers to the head end which is 0·04 mm. across, and to the tail, where the diameter is 0·0084 mm. The cuticle is striated, the tail end slightly curved. Anal orifice was not made out. Alimentary canal simple. The double uterus was observed to commence at a distance of 4·35 cm. from the tail end in a sacculated extremity. The ova, containing coiled up embryos, measure 0·032 by 0·034 mm. Embryos are 0·18 to 0·2 mm. long and 0·001 broad.

The male is smaller and thinner than the female, being on an average 3.14 cm. long, and 0·44 mm. broad. The worm is white and flattened somewhat, has a striated cuticle, and is uniformly tapered towards each end. The diameter of the head is 0·044 mm., and of the tail posterior to the anal orifice 0·028 mm. The head is rounded; tail markedly incurved. The alimentary canal is simple. The anal orifice is at a distance of 0·049 mm. from the caudal extremity. The extreme end of the tail on the concave side is flattened, and here four papillae were made out. The anal orifice itself seems to have two lateral, one post- and one pre-anal papillae on each side. Two unequal spicules—one protruding was slightly clubbed at the extremity, and trumpet shaped at its inner end; the other commencing just within the orifice was much longer than the first, and of much the same shape, but narrower at the point. A narrow, central canal was observed in the former which has a minute opening at its free end.

1. Prout, *British Medical Journal*, January 26, 1901, p. 209.

Life history unknown. The adults occur in pairs in subcutaneous tumours, the purulent contents of which swarm with embryos. These are 0·25 mm. long, by 0·005 mm. broad, have a rounded head, sometimes a double tip. Their tails are sharp and granular. They have no sheath. In stained specimens a V-shaped spot can be made out at the junction of the anterior fifth with the posterior four-fifths.

Filaria labialis. Pane

The female only is known. It is about 40 mm. long, slender, tapering at each end, but slightly swollen at the extreme posterior end. The mouth is surrounded by four papillae. The anus is at 0·5 mm. distance from the posterior end, while the vulva opens at 2·5 mm. more anteriorly. The uterus is double.

Life history unknown. A single specimen only has been seen in a small pustule on the inner side of the upper lip.

Filaria hominis oris. Leidy

Length 140 cm., breadth 0·16 mm. A filiform, opaque white worm, with a simple round mouth, and blunt tail furnished with a short hook 50 μ in length and 12 μ across at the base.

Life history unknown. A single specimen has been found in the mouth of a child. It is thought by Leidy and Leuchart to be an immature *F. medinensis*.

Filaria lymphatica. Treutler

Syn. *Haemularia lymphatica*, Treutler; *F. hominus bronchialis*, Rudolphi. Length, 26 mm.; brownish in colour, speckled with white, almost transparent posteriorly. Body filiform, a little compressed laterally.

Life history unknown. Found in hypertrophied lymphatic glands. Diesing and Weinland regard it as identical with *Strongylus longevaginatus* (*paradoxus*). Railliet suggests it as a male *F. inermis*.

Filaria restiformis. Leidy

A single specimen only found, passed *per urethram*. Length, 65 cm.; breadth, 1·5 mm. Long, uniformly cylindrical body. Cuticle smooth, no transverse striation; head end tapering somewhat, rounded, no appendages. Caudal end incurved, no appendages. No apparent anal or genital aperture. Railliet considers this a pseudo-parasite.

Some Filariae which are Parasites of Animals

Filaria equina. Abildgaard

Syn. *Gordius equinus*, Abildgaard; *F. equi*, Gmelin; *F. papillosa*, Rudolphi; *F. equina*, Blanchard.

A whitish filiform worm tapering towards the extremities especially posteriorly. Cuticle finely striated transversely. Mouth small, round, provided with a chitinous ring, the edge of which has laterally two crescentic lips, and at a point corresponding

to the dorsal and ventral median lines, a simple or indented papilla; behind the ring are four submedian papilliform chitinous spicules.

The male is 6-8 cm. long, has a spiral tail, with four pre- and four post-anal papillae, and two unequal spicules.

The female is 9·12 cm. long, has a slightly spiral tale terminating in a rounded button, in front of which are two lateral conical protuberances. The worm is viviparous; embryos measure 280 μ by 7 μ wide.

The embryos have been observed in the blood of animals found afterwards to contain the adult forms. They are one-seventh mm. in length, and one to three occurred in each drop of blood. They resemble the embryos of *Filaria sanquinis hominis*, but are much smaller.

Life history is unknown, but it is surmised that development takes place in the body of an insect host. The adults have been found in the peritoneal cavity, tunica vaginalis, fallopian tube, pleural cavity, between the dura and pia-mater, in the aqueous humour, in the intestine, and in the liver of horses, donkeys, and mules.

Filaria labiato-papillosa. Alessandrini

Syn. *F. cervina*, Dujardin; *F. terebra*, Diesing. This species resembles the preceding in its appearance and dimensions. Mouth is oblong dorsoventrally, surrounded with a chitinous ring, the edge of which supports four curved projections. On the median, dorsal, and central line, the chitinous ring forms a papilliform spine, markedly double in the female. Behind the mouth are four small sub-median depressions, from each of which a tactile papilla arises. The male is 6-8 cm. long; tail is closely spiral; has three pre-anal, one ad-anal, and five post-anal papillae on each side, and behind these a conical projection. The female is 6-12 cm. long; has a spiral tail, terminating in a number of small blunt points which arise from two lateral conical protuberances. The worm is viviparous; embryos 140 to 230 μ long.

Life history is unknown. The adults have been found in the peritoneal cavity of cattle and deer.

Filaria haemorrhagica. Railliet

Syn. F. multipapillosa, Condamine and Drouilly; *F. multipapilla*, Molin. White cylindrical body, slightly tapering at the extremities, more so behind than in front. Anterior extremity has a retractile cone. The integument is transversely striated. The striations near the anterior extremity become broken, and form elliptical or circular depressions, and a large number of papilliform projections. The mouth is simple, circular.

The male is about 28 mm. long, 0·26 broad; posterior extremity is rounded, there are two unequal spicules, one 680–750 μ long, the other 130-140 μ.

The female has a length of 42–70 mm., and breadth 0·42–0·44 mm.; caudal end rounded: the vulva is near the mouth. The ripe eggs are from 52-58 μ long, 24 to 33 μ wide, and contain an embryo.

The free embryos measure 220-230 μ long by 9-11 μ wide.

The *life history* is unknown. The male and female live together in the connective tissues of the horse and donkey giving rise to hemispherical protuberances about the size of a nut, beneath the skin. These quickly burst and allow blood to escape, after which they subside and appear again in twenty-four to forty-eight hours in other places. Tracts of the worm can be seen in many tissues post-mortem. It is surmised that the embryos are taken up by some insect or crustacean.

Filaria immitis. Leidy

Syn. *F. canis cordis*, LEIDY; *F. papillosa*, *haematica canis domestici*, GRUBY and DELAFOND.

Body white, filiform, a little tapering at each extremity especially posteriorly. Anterior extremity rounded. Mouth terminal, small, simple, surrounded by six small indistinct papillae. Anus near the end of tail.

The male 12 to 18 cm. long, 0·7 to 0·9 mm. broad, with spirally wormed tail bearing two small lateral ridges supported by papillae, four of which are larger than the others—there are three pre- and one post-anal papillae, MANSON[1] however describes the arrangement of papillae differently. Two unequal spicules.

The female is 25-30 cm. long, 1 to 1·3 mm. broad. The tail is short, blunt and curved; vulva is at a distance of about 7 mm. from the mouth. The ova hatch within the uterus: the free embryos measure 285 to 295 μ by 5 μ; their anterior extremities are slightly tapered and end bluntly, the posterior tapers gradually to a fine point. The embryos occur in large numbers in the blood of the infected animal. MANSON observed a certain degree of periodicity, the embryos being most numerous in the peripheral blood at night, not disappearing entirely however during the day.

Life history. The adult parasites are found chiefly in the right ventricle of the heart of the dog, fox, and wolf.

The development of the embryos has been the subject of many investigations. BANCROFT affirmed that he found the embryos in the intestine of *Trichodectes* which had sucked the blood of infected dogs, and supposed these insects to play the part of intermediary host. SONSINO confirmed this, but recognized later that *Trichodectes canis* does not suck blood and that *Haematopinus pilifer* was meant. GRASSI and SONSINO found larvae of Nematodes in the intestine and body cavity of dog fleas, and concluded they were dealing with the embryos of either *Spiroptera sanguinolenta* or of *Filaria immitis*. Subsequently it was found that Spiroptera do not give rise to haematozoal embryos, and it was inferred that dog fleas were the intermediary hosts. Later GRASSI conclusively proved that neither *Pulex serraticeps*, *Haematopinus*, nor ticks (*Rhipicephalus siculus*, KOCH) served as the hosts for *F. immitis*. In the previous

1. Manson, *Medical Times and Gazette*, 1877. Vol. II. page 513. etc.

investigations, he claimed that SONSINO was led astray by the coincidence that *F. recondita* was present in the dogs he examined, the embryos of which were mistaken for those of *F. immitis*. GRASSI then thought the intermediary host to be a crustacean or mollusc.

However in 1900 he[1] describes the development of these embryos inside the mosquito. 'The embryos sucked up by *Anopheles* migrate into the malpighian tubes, where they continue their development behaving more or less like the other blood filariae already known. The larvae, arrived at maximum development, abandon the tubes and enter the general body cavity leaving behind the old cuticle: there they progress towards the head and collect there rapidly in the prolongation of the general body cavity within the labium (called also the inferior labium), occasionally in the palpae.' In their experiments these authors seem to have allowed a period of thirteen or fourteen days for the complete development of the embryos in *Anopheles*. They do not appear, however, as far as we have been able to ascertain to have carried out the infection of healthy dogs by the bites of infected *Anopheles*. One experiment is described, undertaken on July 19, 1900, in which a healthy dog was injected subcutaneously with larvae, collected in a drop of normal saline solution, from the labium of two infected *Anopheles*. At the post-mortem on August 4th (a period of *sixteen* days) there was found 'in the subcutaneous tissue near the genitals, a very small female filaria which must be judged *Filaria immitis*, still immature. We were able to preserve only its anterior half sufficiently for diagnosis.' This does not seem to us very satisfactory; details of the appearance and anatomy of this anterior half of an immature *Filaria immitis* not being given.

Filaria recondita. Grassi[2]

The female only is known. This is about 3 cm. long, 0·178 mm. broad. The transparent body tapers towards both ends, more especially posteriorly. The integument is nonstriated. The anterior extremity is obtuse, bears at least four very small papillae close to the buccal orifice. Posterior extremity is also blunt, and has three papillae, one terminal and two lateral, and also several small papilliform projections. The mouth is followed by a very short cylindrical oesophagus, somewhat less than 2·5 mm. long. The anus is at a distance of 228 μ from the tip of the tail. The uterus is double, the vulva at a distance of 840 μ behind the mouth.

Life history. Up to the present only a single female specimen (which was immature, containing neither embryos nor eggs) has been met with. It was found coiled up but not encysted in the adipose tissue near the hilum of the dog's kidney. The embryos have been studied by GRUBY and DELAFOND, LEWIS, MANSON, GRASSI, SONSINO, and others, in France, China, India, and Italy.

1. Grassi and Noé, *British Med. Journal*, 1900. Nov. 3, p. 1306.
2. In a footnote in his article on 'Filariasis' in the *Encyclopaedia Medica*, Vol. III, Nuttall says: 'Sonsino (personal communication, December, 1899) considers it doubtful that this is a 'good species,' the determination having been made upon a single female specimen.'

GRASSI and CALANDRUCCIO[1] traced out the development of the embroyos in *Pulex serraticeps* (of the dog and cat), *Pulex irritans* (of man and dog), and *Rhipicephalus siculus*, KOCH (a dog-tick). They describe the following stages:—

First Stage. Embryo found in the blood of dogs, and in the intestine and body cavity of fleas. Length 280 μ, breadth 5 μ. Body slightly thinned in front, but ending bluntly: behind it tapers and ends in an almost hair-fine point. It is smaller than the embryo of *F. immitis*, and possesses the characteristic that they fix their oral end to the coverglass. At the front end can be made out a fine canal, representing the oesophagus. In those which have reached the body cavity there can be made out a certain trace of the intestinal tract and of the anus. The embryo executes snake-like movements.

Second Stage. Found in the fat cells, seldom free in the body cavity. The larvae of the previous stage first shorten without thickening, then thicken and finally lengthen. The cells of the larvae are larger, and the organs more distinct. The body is cylindrical, and in front has a finger-like papilla 5·6 μ long, covered with cuticle somewhat thickened at the free end, and appearing as though filled with a clear liquid. Long pointed tail. Parts of the alimentary tract are becoming differentiated. The genital apparatus is just appearing. The worm has no movement.

Third Stage. A moulting of the cuticle takes place either in the cell or when free in the body cavity. Length reaches 1·5 mm. The front end is blunt, the papilla of the previous stage disappears. Hind end has three papilla, one terminal dorsal, two other almost terminal, and ventral. The fine point of the tail has disappeared. Further development of the alimentary organs—mouth opening has four papillae. The worm shows active eel-like movements.

Fourth Stage. Only once seen. The larva was encysted, and was considerably larger and thicker. Genital apparatus developed. The tail, besides the papillae, bears a little process (as in adult).

Stages three and four are similar to the adult, and much further development cannot take place.

Attempts, however, to infect dogs with infected fleas failed.

Filaria irritans. Rivolta

Syn. *Dermofilaria irritans.* This name is given to a nematode larva, which measures about 3 mm. in length; its head is slightly marked off by a neck from the body; the tail tapers and terminated in a blunt notched point. The mouth is round, and appears to be provided with lips. At a little distance from the head end an opening is seen. The anus occurs at the point where the body tapers into the tail. The integument bears fine transverse striations.

The *life history* is unknown. These larvae are found in the 'summer sores' or 'granular dermatitis' or horses and donkeys.

1. Grassi and Calandruccio, *Centralblatt für Bakteriologie*, 1890, vii, 18-26

Filaria evansi. Lewis

A description of this species[1] of which the male and female are known, is not procurable. The worms were found in the lung and mesentery of a camel at Madras, the pulmonary arteries being obstructed by masses of tangled worms—the blood containing number embryos similar to those of *F. bancrofti.*

Filaria lachrymalis. Guret

Syn. F. bovis, BAILLET. *F. palpebrarum*, RAILLET. A whitish cylindrical worm, slightly tapering at each end. Cuticle transversely striated. Mouth small, simple, followed by a cylindrical buccal cavity. Anus almost terminal. Male 10-14 mm. long, tail bowed, has two very unequal spicules. Female is 15-24 mm. long, has a straight conical tail. Vulva about 1 mm. from anterior end. Ova ellipsoid, hatch inside the uterus. Embryos 210-230 μ long.

Life history unknown. The adults live in the lachrymal-duct of cattle.

Filaria palpebralis. Wilson

Has a white cylindrical body, slightly tapering at each end. Cuticle has fine transverse striations. Small, simple mouth. Anus almost terminal. Male 8-12 mm. long, tail curved ; bears three pairs of post-anal of papillae and two unequal spicules. The female is 14-22 mm. long, and has a straight conical tail ; the vulva is at 0·60-0·70 mm. from the anterior end. Ova are ellipsoid hatch under the uterus. Embryos have a length of 120-170 μ.

Life history unknown. The adults have been found in the excretory ducts of the lachrymal glands and under the eyelids of the horse.

Filaria osleri. Cobbold

Syn. *Strongylus bronchialis canis*, OSLER.

Body filiform ; mouth surrounded by two or three lips behind which are three unequal papillae ; pharynx swollen. The male is 5 mm. long and has a rounded posterior end ; and two unequal curved spicules. The female is 9-15 mm. long, tapers at each end ; anus almost terminal ; vulva immediately in front of anus ; the worm is ovoviviparous.

Life history unknown. The adults were found by OSLER to be the cause of an epizootic bronchopneumonia in dogs at Montreal. RABE and RUMBERG had previously observed the worm in small nodules in the mucous membrane of the respiratory passages, each nodule containing several male and female worms.

1. Lewis. Remarks on a Nematoid Haematozoon discovered by Dr. Griffith Evans in a Camel. *Proceedings of the Asiatic Society of Bengal*, 1882, p. 63.

Filaria clava. Wedl

The female only is known—length 16-18 mm., breadth 0·33 mm. Body filiform and of uniform thickness throughout almost the whole length. Head end conical; posterior end rounded and bulbous. Mouth simple, small. Anus in a groove at the bulbous end. Vulva at 1·25 mm. from the anterior end. Ova 36 μ by 24 μ contain a coiled-up embryo. Embryo 84 μ long, 6 μ wide, thin rounded anterior end, pointed posterior end. Found in the peritracheal connective tissue of the domestic pigeon.

Filaria mazzanti. Railliet

The female which alone is known is 25 mm. long, 0·25 wide; has a rounded anterior end, conical posterior end. Mouth simple, round. Anus terminal. Vulva triangular, 213 μ from anterior end. Viviparous. Found under the skin of the neck of a pigeon, whose blood contain embryos some 185 μ long with slightly pointed tail, the others 142 μ long with blunt tails.

Filaria uncinata. Rudolphi

Syn. *Spiroptera uncinata*, RUDOLPHI; *Dispharage à queue crochue*, RAILLIET; *F. uncinata*, RUDOLPHI. Mouth has two lips with six papillae. The four sinuous cutaneous bands (characteristic of the *Dispharagi* RAILLIET[1]) reach to within 2 mm. of the anterior end. On each side of the body a double longitudinal series of small spines extends almost to the caudal extremity; in front, the spine ridges reach the dorsal surface and approach the mouth between the cutaneous bands.

The male is 9-10 mm. long; the tail shows straight lateral alae with vesicular edges. Four post-anal papillae; the pre-anal five or six side by side, or seven or eight; the principal spicule is long, incurved and dilated at its free extremity; the other is thick and short.

The female is 15 to 18 mm. long; vulva is at about 1 mm. from the caudal extremity, which is curved.

Life history. This has been worked out by HAMANN[2] in *Daphnia pulex* (RICH). The adults occur in the oesophagus and ventriculus of geese and ducks in tubercles of different sizes which contain worms up to about 10 mm. in length, coiled together. The disease attacks the younger animals of late generations; those of the first brood are unaffected, explained by the course of the development of *Daphnia*. This crustacean multiplies the whole year round, but mostly in the hot summer months, especially of July and August. The mature worms give rise to embryos which wander out of the tumour and may, either, come out by the oesophagus and mouth, or, more usually passed through the intestine, and escape

1. Railliet, *Zool Medic et Agric.* Paris, 1895, p. 542.
2. Hamann, *Central. f. Bakt. u. Paras.*, 1893, xiv, p. 555.

per rectum. They are then taken up by *Daphnia* ; bore through the intestine and lie in the body cavity, reaching 1·7-2 mm. in length. They show a typical mouth with six papillae and a 'vestibulum' ; only the generative organs are lacking. They are then swallowed by ducks and bore into the wall of the stomach and oesophagus. STOSSICH does not include this worm in his list of *Filariae*.

Filaria picae mediae. Manson [1]

The male and female were found coiled up in a small white tubercle in the pocket of the semi-lunar valve; the worms were encysted, and a minute opening in the covering of the cyst may be present.

The male is about 18·5 mm. long ; diameter at the neck about 0·06 mm. ; greatest diameter about 0·18 mm., of alimentary canal about 0·06 mm., and of spermatic tube 0·08 mm. The tail is strongly incurved. Two spicules. One or two minute caudal papillae. The tail is blunted and slightly lobed and tapers down from the body. The mouth is simple ; oesophagus straight about 0·5 mm. long, terminates in the alimentary canal by a gradual dilatation. Alimentary canal is straight, and filled with a dark granular material. The integument is covered with minute bosses or tubercles, largest about the middle of the animal, less marked towards the head and tail ends.

The female averages about 37 mm. in length ; greatest diameter of unimpregnated specimen about 0·2 mm. ; impregnated about 0·3 mm. The anus is at about 0·12 mm. from the caudal extremity. The vagina is infundibuliform, and opens at 0·25 mm. from the mouth. Mouth, oesophagus and alimentary are similar to those of the male. Expressed embryos measure 0·12 mm. long by 0·004 mm. broad. They have no sheath ; tails are truncated.

In the blood of this bird, MANSON found embryos apparently of two kinds, one about 0·1 mm. long ; the other about 0·22 mm. ; intermediate sizes were also present. The smaller were languid, the larger very active in the movements. A jerking pouting oral movement was seen in both. Tails sharp and pointed.

Filaria corvi torquatis. Manson

The adults occur in the right ventricle, pulmonary artery and its branches.

The male is about 0·3 mm. long ; greatest diameter about 0·16 mm. ; diameter of neck 0·06 mm. Length of oesophagus 0·6 mm. ; diameter 0·04 mm. The body is smooth and very transparent. Mouth simple ; spicules double. No papillae. Tail tapers to a blunt extremity. Anus close to end of tail.

Female 18-20 mm. long ; diameter 0·27 mm. Vagina opens 0·25 mm. from mouth.

The blood of the bird contained two dissimilar embryos ; the larger 0·21 to 0·25 mm. long, and 0·008 mm. broad ; the smaller 0·13 mm. long, 0·004 mm. broad.

1. Manson, *Journal of the Quekett Club*, vol. xi, 1880, p. 130.

The former showed active, lashing, free, vigorous movement; the latter languid, slow wriggling. Oral movements were distinct; there were four papillae round the mouth. The tail of the larger tapered and was pointed; of the smaller, tapered slightly, was truncated, and a thin skin extended like a loose bag or hood from the head end.

MANSON also gives meagre descriptions of haematozoal embryos in *Gracupica nigricollis* and *Goura coronata* (Malay Archipelago).

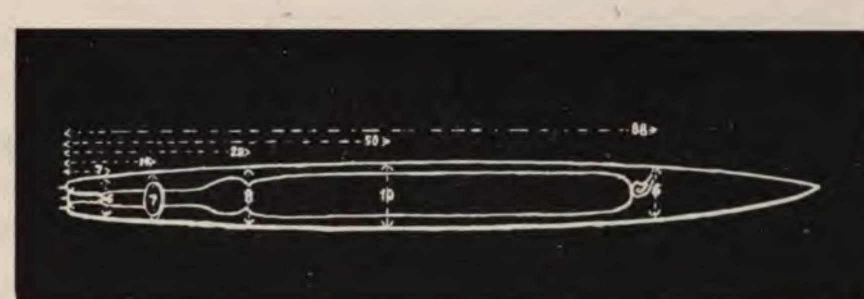
STOSSICH[1] describes in his monograph 212 species of *Filariae*—we have arranged a complete list of these from his work, giving their hosts, and sites, and also the literature referring to each. This list will be found in the Bibliography.

1. Stossich, *Filarie e Spiroptere*, Trieste, 1897.

III. *AVIAN FILARIAE.*—New Species

This description of new blood filariae discovered in West African birds of different species includes the account of eight new species, of which the adult forms generally both male and female were found, as well as the blood embryos, and also of three species in which blood embryos alone were met with.

With a view to obtaining some uniformity in the descriptions and measurements of Nematode worms in general, Cobb[1] has devised an ingenious formula, for the account of which we are indebted to Shipley[2], in which measurements of different parts appear as percentages of the whole length of the body. The following diagram explains the nature of the formula, which, however, should be used with caution since it rests on the assumption, as Shipley[2] points out, that the proportions of the various parts of the body are constant in different individuals, and it is by no means certain that this is the case.



In the diagram, 6, 7, 8, 10, and 6 are the transverse measurements, while 7, 14, 28, 50, and 88 are the corresponding longitudinal measurements. The formula in this case is

$$\frac{7 \quad 14 \quad 28 \quad 50 \quad 88}{6 \quad 7 \quad 8 \quad 10 \quad 6}.$$

The unit of measurement is the one-hundredth part of the length of the worm, so that the measurements are therefore percentages of the length. The measurements are taken with the animal viewed in profile; the first is taken at the base of the oesophagus, the second at the nerve ring, the third at the cardiac constriction, the fourth at the vulva in the female and at the middle in the male, the fifth at the anus.

1. Cobb, *Macleay Memorial Volume*, Sidney, 1893, p. 252; and *Proc. Linnean Society*, N.S.W. Second series, Vol. V, 1890, p. 449.
2. Shipley, Harmer and Shipley, *Cambridge Natural History*, Vol. II, *Worms*, etc., p. 138.

This plan will be followed as nearly as possible in the following descriptions :—

Filaria cypseli. Nov. Sp.

Definitive bost—Cypselus affinis. The West African swift. The infected birds were found to have built their nests among the rafters supporting the verandah of the telegraph station of the African Direct Telegraph Company at Bonny, Southern Nigeria ; and in the neighbouring palm trees.

Site. The adult filariae were found in the subcutaneous tissues of the head and neck. In one bird six worms occurred, four of which were mature females, one an immature female, and the sixth a mature male ; in another two females and one male. Some came off with the skin on stripping the scalp ; two were found in the neck, one of them extending as far down as the middle of the back. They were not coiled up but lay more or less straight among the subcutaneous tissues. They were observed to move in the tissue with a slow sinuous motion backwards and forwards, and could be kept alive in normal salt solution for about ten hours.

The adult worms are very long and thin, white in colour. The cuticle shows faint transverse striations. The female has an average length of 25·3 mm.—the length varying in our specimens from 24·0 to 26·7 mm. (the immature female measured only 16 mm. long). The breadth of the body is 0·22 mm.

$$\text{Cobb's formula is : } \frac{—,\ 0{\cdot}54,\ 1{\cdot}7,\ 2{\cdot}5,\ t,^{*}}{—,\ 0{\cdot}8,\ 0{\cdot}88,\ 0{\cdot}84,\ 0{\cdot}8,},$$

The head end [plate I, fig. 2] is somewhat bulbous, and has the shape of a short cone slightly flattened at the apex—which is the position of the oral orifice. On the rim of the slightly flattened area are four minute papillae. The oral orifice is placed centrally—no buccal appendages can be made out. The buccal cavity is continued backwards into a thick-walled narrow-lumened oesophagus, which is 0·45 mm. long, bulbous posteriorly, and distinctly marked off by a constriction from the rest of the alimentary tract. What appears to be the nerve collar or commissure crosses the oesophagus at a distance of about one quarter of its length from the anterior end. The gut is continued almost straight down the length of the worm, curving only from side to side, and ending at the terminally placed anus. The position of the anal orifice is indicated by a depression placed slightly subterminally. The tail end does not taper, but is slightly swollen at the extreme end, which is very abruptly rounded off. (Plate I, fig. 3). The vaginal orifice is situated at a distance of 0·7 mm. from the anterior end, and is placed at the centre of a small conical papilla. Two minute pre- and two post-vaginal spines can be made out (there may be six in all). The vagina which has thick muscular walls is directed generally backwards, but according to the state of engorgement of the uterus it may first go a little backwards and then

* *t* denotes that the position of the anus is terminal.

make a loop forwards before turning; it coils backwards to a point about one-quarter down the length of the worm and receives the two horns of the uterus. In ripe specimens the vagina is seen packed with numerous outstretched embryos arranged longitudinally. The two uterine horns make many longitudinal coils and twists round each other, which may extend up as far as the junction of the oesophagus and intestine and backwards to the posterior end of the worm. Near their termination they narrow considerably and end in long blunt nodular extremities in the posterior quarter of the worm. In the mature worm the contents are first granular in the narrowed terminal portion ; the granules increasing in size further on until distinct ova are made out. Beyond this they contain embryos coiled up in the vitelline membranes which, when the embryos have straightened themselves out, are seen to form the embryonic sheaths. In some of our specimens many embryos enveloped in their characteristic sheaths have escaped. These and the ova are found to have the following measurements : —

Length of ovum containing coiled up embryo	36 μ
Breadth ,, ,, ,, ,,	23 μ
Length of freshly hatched embryo	76·5 μ
Breadth ,, ,,	8·2 μ

The male is much smaller than the female. It is found in similar positions, and in general characters resembles the female, although it is shorter and thinner : it is characterised in preserved specimens by the strongly incurved tail, which makes two almost complete turns. Its average length is 7·5 mm., its breadth 0·15 mm.

$$\text{Cobb's formula}: \frac{\text{—, } 1{\cdot}02,\ 4{\cdot}07,\ 50,\ t,}{\text{—, } 1{\cdot}63,\ 1{\cdot}63,\ 1{\cdot}02,\ \text{—}}$$

The head end (plate I, fig. 4) is similar in shape to that of the female. The length of the oesophagus is 0·32 mm. There is a distinct cardiac constriction. The anal orifice appears to be placed, not exactly terminal, but rather on the ventral surface. There are three pairs of pre-anal and one pair of post-anal papillae; the posterior two of the pre-anal series are larger, and are united by low ridges with the corresponding papillae of the opposite side. There are two spicules of unequal length—not extruded in our specimens. The tail end (plate I, fig. 5) does not taper, the extremity somewhat resembles that of the female, except that dorsally it is not so abruptly rounded off.

The embryo. The habitat of the embryo seemed to be essentially the lymph. In the process of the preparation of our specimens, it was often observed that in those made from the blood of the claws and legs by puncture of a small blood-vessel, one only, out of many slides, was occasionally found to contain very few embryos; many contained none at all. Moreover, we never found any embryos in the heart's

blood. On more careful examination it was found that the claws appeared somewhat oedematous, and by careful manipulation we were able to obtain specimens of the serous fluid, which contained large numbers of the embryos.

The embryo (plate I, fig. 1) in the fresh condition as seen in lymph and some blood preparations was $84{\cdot}7\,\mu$ long. The breadth of the sheath of the embryo, $12{\cdot}78\,\mu$; of the embryo itself inside its sheath, $7{\cdot}9\,\mu$. When fresh the embryos exhibited a slow sinuous progressive movement: while, inside the sheath they were much more active. The two ends of the worm continually moved about, so that the tips seemed always in contact with the inner surface of the bluntly conical end of the sheath—the ends never being observed retracted from the sheath. This movement of the extremities inside the sheath, which appears a little too short for the embryo, causes the body of the embryo to be thrown into two curves, the sheath crinkling a little opposite the concavities of the curves. Ecdysis was not observed.

Both extremities of the embryo are bluntly rounded. At the anterior extremity is a short stout conical papilla from the apex of which projects a short thick spine which is always closely applied to the inner surface of the rounded end of the sheath. There is no prepuce, but a distinct ridge marks off the body from the papilla: neither spine nor papilla was observed to be withdrawn. Under high powers a central line appears to run down from the papilla into the body. The anterior portion of the body of the embryo tapers very slightly. The contents are finely granular, a larger more refractile granule appearing at a point at about a quarter of the length from the posterior end. At this end the worm has a short rather broad, highly refractile tubercle which is always in contact with the sheath, and moves from side to side along the concavity of the end of the sheath.

In fixed and stained specimens, in all of which the embryo is found shrunk in various degrees inside its capsule, the length varies from 75 to $84{\cdot}7\,\mu$. The nuclei of the very small cells are evident, but indications of **V**-shaped or other shaped spots are very indefinite and irregular.

Filaria spiralis avium. Nov. Sp.

Definitive hosts.—*Hyphantornis aurantius.*
Cyanomitra reichenbachi.
Muscicapidarum. Sp. dub.
Pyenonotus barbatus.
Sitagra brachyptera.
Vidua principalis.
Cinnyris fuliginosa.
Cypselus affinis.

Site. The adult worms were always found in swellings about the feet and ankles of these birds. The infected birds were easy to detect by the presence of small, soft, subcutaneous tumours in these positions; the skin over these tumours was stretched, and the superficial veins appeared dilated.

The small nodules occurred in various positions: for example in one bird—on the right foot, a tumour on the upper surface of the second phalanx of the first toe, and in a similar position on the second toe; a third on the under surface of terminal phalanx of the fourth toe; another over the distal end of tarsus. In the left foot—a large swelling under the distal end of the tarsus; two on the first toe, one at its extreme base on the under surface, the other on the under surface of the terminal phalanx; and one on the lateral outer surface of the fourth toe. In another bird, one tumour was found higher up, in the tarsus among the tendons; others just at the base of the claws, under the insertion of the flexor tendons.

The worms occupied cysts in the positions mentioned, from two to ten worms in each cyst, which seemed to be intimately connected with the sheaths of the tendons. The worms were coiled together inside the cyst; the whole clump of them being easily turned out on slitting up the tumour, with a mass of yellow coloured jelly-like fluid, which surrounded the worms.

The worms in the cysts varied in colour from pale yellow to brown, the younger worms being generally brown. Some were considerably larger than others. They have a decidedly corkscrew shape, the screw having two to four turns according to the length of the worm. Introduced into normal salt solution the worms retained their corkscrew shape and moved for some time with a sluggish corkscrew motion. The shape is kept in preserved specimens. The screw of both male and female worms is a right-handed one, these facilitating the arrangement of a large number into the smallest space.

The female :—The total length varies from 4·4 to 8·4 mm., the central breadth about 0·34 mm. The worm makes three or four coils.

$$\text{Cobb's formula}: \frac{—,\ 1{\cdot}8,\ 10{\cdot}2,\ 4{\cdot}3,\ 99{\cdot}2}{—,\ 1{\cdot}2,\ 2{\cdot}8,\ 1{\cdot}7,\ 0{\cdot}94},$$

The anterior and posterior portions of the worms beyond the beginning and end of the spiral are somewhat flattened; the anterior portion is longer than the posterior, and more sharply pointed. The cuticle is thick, transparent, and yellowish in colour: over the extreme ends it is thin. Laterally in the anterior portion the cuticle is thickened, the two lateral thickenings being continued down throughout the length of the worm, so that in optical sections of the convexities of the spirals they appear as knobs, of the concavities as thickenings.

The anterior endo the worm (plate II, fig. 2) which is tapered from the point of junction with the spiral proper, is rounded; there is a slight narrowing for a neck. The position of the oral orifice is marked as a small dent in the cuticle. No

papillae nor tubercles are evident. The long oesophagus, extending down beyond the vaginal orifice, is about 0·75 mm. long, and bears a narrow bulb marked off from the intestine by a slight constriction often hidden by the vagina. The gut extends along the whole length of the worm, and terminates at the anus on the ventral surface just in front of the posterior extremity of the worm. The anal orifice is surrounded by five delicate lips giving a rosette appearance. Side view the position of the orifice is marked by a slight baying (plate II, figs. 3 and 4). The vulva is at a distance of about 0·33 mm. from the head end, and appears to open ventro-laterally. The vagina runs, for a short way, directly backwards, makes a coil towards the head end, and runs down. The first portion is very thick-walled with small lumen ; beyond this the walls get thinner, and the lumen is seen packed with stretched-out embryos. The vagina receives the two horns of the uterus, which coil and twist round each other, and extend to the posterior end of the worm. Each horn has muscular walls at its entrance into the vagina; the muscular walls get thinner and the lumen narrows somewhat in diameter until a kind of neck is reached following a distinct bulbous swelling, in the region of which the muscular walls are much thicker, forming a sort of 'pylorus.' Beyond this the tube again narrows, the walls are very thick, so that only a narrow lumen is apparent. At the junction of this thick-walled tube (oviduct) with the bulbous swelling (uterus), the former projects into the cavity of the latter to form a papilla with an opening at its centre. Beyond this thick-walled oviduct is the ovary—a long wider thin-walled portion, which further on narrows considerably, becoming cord-like, and ends in a terminal bulb, immediately in front of which is a small swelling. The length of the ovary is about 1·7 mm.—of the oviduct 0·9 mm. The total length from vulva to the end of the ovary is about 26 mm.

Mature ova—spherical cells having large rounded nuclei and distinct nucleoli—are found in the large dilated proximal portion of the ovary. The narrow-lumened oviduct is empty. The cavity of the uterus near its junction with the oviduct, and for some distance down contains innumerable spermatozoa surrounding several ova. Beyond this, the uterus contains ova in all stages of development.*

Length of ovum containing embryo		39 μ.
Breadth	,, ,, ,,	27 μ.
Length of embryo with its sheath		236 μ.
Breadth	,, ,,	5 to 6 μ.

The male is similar to the female in appearance but considerably smaller ; it makes two or three spirals. The tail end has its tip curled ventrally. Length of worm 3·4 to 3·7 mm. ; breadth 0·2 to 0·3 mm.

$$\text{Cobb's formula } \frac{-,\ 2\cdot3,\ 15\cdot7,\ 50,\ 98\cdot6}{-,\ 1\cdot9,\ 3\cdot8,\ 4\cdot6,\ 1\cdot5};$$

*A more detailed account of the histology of the reproductive and other systems of these worms will form the subject of a subsequent article.

The anterior end resembles that of the female but is smaller. The oesophagus is 0·57 mm. long (in one very transparent specimen only, a distinct cardiac constriction could be made out). The anal orifice is at a point 0·08 mm. from the tip of the tail (plate III, figs. 1 and 2). Four pre-anal and three post-anal papillae on each side could be made out; the two last post-anal being very small. The genital orifice is in the median line at the apex of a slight raised prominence. On each side of this prominence are two cuticular expansions, bearing the papillae and forming continuations of the lateral cuticular ridges. There are two curved unequal retractile spicules, the ventral of which, shorter than the dorsal, appears to be hollowed out on its dorsal surface for the latter's reception. The dorsal spicule is rod shaped and ends in a round knob. The other seems to widen at its deeper end and bends round the sides of the dorsal. The spermatic canal runs up the worm from the neighbourhood of the base of the spicules as a single narrow tube which soon widens to fill up almost the whole of the body cavity. A short distance from the head it becomes somewhat narrower, and ends after making a few turns in this region.

The embryos are found in large numbers in the peripheral and in the heart's blood. They have a sheath which is a long narrow cylinder with rounded ends. In fresh specimens the embryos exhibit a simple snake-like lashing movement, progressing forwards and backwards, and also a backward and forward motion inside the sheath. Some were seen to coil themselves up closely. The worm with its sheath (plate XIII, fig. 2) has a uniform thickness, except at the posterior end where it suddenly diminishes into a wall-marked 'tail.' The length of the worm inside the sheath was 208·6 μ, breadth 1·7 μ. In the living specimens two longitudinal lines of fine refractile granules can be observed, one about the junction of the anterior and middle thirds, the other about the junction of the middle and posterior thirds. The head end is rounded, no definite prepuce nor spine could be made out, beyond a highly refractile 'glans'-like tip. At the tail end the width of the worm suddenly diminishes at a distance of about 6·5 μ from the extreme tip to about 2 μ. The tail comes off eccentrically.

In stained specimens (plate III, fig. 5) no characteristic position is acquired. The average length of the worm, which varies considerably, is 216·8 μ. The column of small nucleated cells forming the body, at the head end is bayed out so that the end appears bifid. Five 'spots' (V- or otherwise-shaped) can be made out; their numbers however varies from three to five.

On examination of a number of embryos the distances of the spots from the end of the worm relatively to its length proves to be fairly constant, and we shall adopt throughout this work a method similar to Cobb's to indicate their positions; expressing the distances of the middle of the spots from the anterior end in percentages of the total length:

1. A transverse slit: distance 24·5 per cent. of length; sometimes not seen.

2. A clear sometimes lateral, sometimes transverse spot; distance 35.3; constant.
3. A long space in which the nuclei are loosely arranged, often anteriorly and posteriorly ending in a clear space, with the nuclei more densely arranged in between; distance 66·7; constant.
4. A small spot only occasionally seen, at 76; sometimes merges into the third spot.
5. A very small lateral spot sometimes absent; distance 89·5.

Filaria fusiformis avium. Nov. Spec.

Definitive hosts : *Spermestus cucullatus.*
Hyphantornis. Sp. incert.
Hyphantornis aurantus.

Sites. The adult forms were found in the mesentery at the under surface of the liver and in the lung. The collection comprises two males, four females, and three immature worms: of which two were found in one bird; a single one in another; another single one in a bird whose blood contained also embryos of *F. spiralis* and *F. opobensis*; the others in a fourth.

The female is a long whitish worm, tapering gradually for some considerable distance at each end. The length varies from 15·8 to 25·5 mm.; breadth about 0·28 mm.

$$\text{Cobb's formula}: \frac{\text{—},\ 0{\cdot}67,\ \text{—},\ 1{\cdot}6,\ \text{—}}{\text{—},\ 0{\cdot}27,\ \text{—},\ 0{\cdot}33,\ \text{—}}$$

The cuticle is thin, smooth and transparent, somewhat thickened at the head end. The worms are all very opaque, so that but little of the internal anatomy can be made out in preserved specimens. At the anterior end (plate IV, fig. 2) which tapers to a breadth of 0·05 mm., is a slight appearance of a somewhat narrower neck. The oral orifice is terminal; no papillae nor spines apparent. The vulva is at a distance of about 0·43 mm. from the anterior end. The anus is terminal. The posterior end also tapers considerably to 0·05 mm., and is then abruptly rounded off (plate IV, fig. 3). The ovum measures 27·7 μ by 24·7 μ, and the length of the embryo inside the sheath in the preserved condition is 120 μ. The male, one specimen only of which is suitable for description, is 11·8 mm. long, 0·16 mm. broad.

$$\text{Cobb's formula}: \frac{\text{—},\ \text{—},\ \text{—},\ 50,\ 0{\cdot}88}{\text{—},\ \text{—},\ \text{—},\ 13{\cdot}8,\ 0{\cdot}47}$$

The cuticle shows no obvious striation. The head end resembles that of the female in shape; it is provided with four small tubercles round the oral orifice. The posterior third of the worm tapers and coils, the coiling increasing towards the end of the tail. The anal orifice opens at a distance of about 0·14 mm. from the tip of the tail. There are two unequal spicules. The specimen is too opaque to make out any further internal anatomy.

The *embryos* are found in the peripheral and in the heart's blood. Its length in the fresh condition is 117 μ, breadth, 3 μ. It has a very marked sheath which can often be seen trailing in front or behind the worm in the fresh specimen (plate XIII, fig. 3). The embryos move backwards or forwards with snaky movements, and also rush very energetically forwards and backwards inside their sheaths. The head end is rounded and has a six lipped prepuce through which a conical papilla can be observed to be protruded; this bears at its apex a fine projecting spine. The papilla and its spine can be retracted within its sheath—an action very actively performed in fresh microscopical preparations. The body contents appear finely granular; at the junction of the anterior and middle thirds is a highly refractile spot. The tail end tapers very slightly and ends bluntly rounded. In stained specimens (plate IV, fig. 4), the length of the embryo is 86·0 μ; the length of the sheath beyond the worm proper both anteriorly and posteriorly varies enormously. The head end shews generally only a looseness in the arrangement of the small cells—sometimes a baying.

Spots.—1. A narrow transverse slit—fairly constant; distance 28·6.

2. A lateral rounded bay—not always present; distance 40·7.

3. A clear band across the worm—constant; distance 69·2.

4. A small lateral slit—only occasionally seen; distance 90·0.

Spots 2 and 4 are always on the same side of the worm.

Filaria spiralis avium major. Nov. Sp.

Definitive hosts: *Hyphantornis.* Sp. incert.
Sitagra brachyptera.
Hyphantornis aurantius.

Site. In *Sitagra brachyptera* the adult worms were found in a thick walled cyst on the right leg situated deeply under the tendons on the bone. The cyst contained one small (male) and two large (female) worms. This bird also contained embryos and adults of *F. spiralis*. Although similar in appearance and site the two worms are quite distinct, the female of *F. spiralis major* being three or four times the length of the female of *F. spiralis*—and moreover they have different embryos, a fact conclusively demonstrated by the rupture of the uterus in each case and the examination of the contained embryos.

The female, spiral in form, has nine turns. The spiral is right-handed. The total length is 17·3 mm., the central breadth 0·43 mm.

$$\text{Cobb's formula: } \frac{-,\ -,\ —,\ 2{\cdot}4,\ t}{-,\ —,\ -,\ 0{\cdot}64 —}$$

Similarly to *F. Spiralis*, there is an anterior and posterior portion beyond the spiral, the anterior of which is the longer. The cuticle at the anterior end is thin, but thicker at the posterior end. Laterally the cuticle is thickened, and along the

convexities of the worm is seen to be distinctly striated transversely. The outer lateral ridge bears a number of flattened transparent nodules, the distribution of which seems to be irregular, in places appearing to be grouped into twos, threes, or fours. The striations of the cuticle become spread out in the nodules. The striations are less distinct along the other lateral border of the worm. The anterior portion (plate V, fig. 2) tapers considerably; its extremity is rounded; the oral orifice is central and terminal--no papillae or other appendages could be made out. The anus is in a position similar to that of *F. spiralis* (plate V, fig 3). The vagina opens at a distance of 0·45 mm. from the anterior end. The opacity of the worms makes it difficult to observe further the internal anatomy. The nodulated cuticle is very characteristic.

The male is much shorter and thinner than the female and has six coils. The tail end is markedly incurved. The length of the worm is 9·0 mm., breadth 0·125 mm.

$$\text{Cobb's formula } \frac{—,\ —,\ 3{\cdot}4,\ 50,\ 99{\cdot}2}{—,\ —,\ 0{\cdot}93,\ 1{\cdot}4,\ 0{\cdot}46}$$

The cuticle is ridged, knobbed, and striated, similarly to that of the female. Beneath the lateral ridges in the musculo-cutaneous structure is a dark brown granular pigmented layer: the pigment, apparently intracellular, is regularly interrupted by what appear to be large unpigmented nuclei. The head end (plate V, fig. 4) is similar to that of the female. The oesophageal bulb is very indistinctly marked. The anal aperture is at a distance of 0·058 mm. from the tip of the tail, and is at the centre of a low flat papilla. Three pre-anal, and two post-anal papillae could be made out on each side, the former being very small and close together, almost continuous with one another. There are two unequal spicules, their terminal extremities have a rosette appearance. The origin of the reproductive tube can be seen to commence as a thin single tube coiled about the neighbourhood of the commencement of the intestine, which increases in size to fill almost completely the whole body cavity up to the last coil of the worm where the tube becomes thinner and its walls more muscular till it ends at the rosette horns of the spicules. The tail end differs from that of *F. spiralis* having no expansions of the lateral cuticular ridges, these disappearing altogether as the tail is reached. The tip is bluntly rounded off on the dorsal surface. (Plate V, fig. 5).

The *embryos* are found in peripheral and central blood. In the fresh condition they measure 141·7 μ in length, 6.5 μ in breadth. They exhibit forward and backward sinuous progressive movements. They have a well marked thick cuticle which shews distinct transverse striations (plate XIII, fig. 4). They taper very slightly towards the anterior end, very abruptly posteriorly, so that this end resembles in shape the point of a wire nail. The head end is bluntly rounded and has a small clear area—no prepuce nor spine could be made out. At the tail end the cuticle is well seen.

In stained specimens (plate VI, fig. 1) the cuticle is also well marked. The worm measures 119·3 μ in length. The embryos on fixing take up no characteristic position. At the head end the cell column is bifid. Four 'spots' are generally made out:

1. Slit like, at a distance of 29·2 per cent. of total length.
2. A small lateral bay; distance 42·6.
3. An oval-shaped spot occupying the breadth of the worm, containing only a few small nuclei; distance 64·4.
4. A small lateral break; distance 89·9.

The two lateral spots (2 and 4) are on the same side of the worm. All the spots are constant, but the first and second are sometimes badly marked.

Filaria falciformis. Nov. Sp.

Definitive Host: *Cinnyris fuliginosa.*

Site. The subcutaneous tissue of the back of the head, dorsum of wing, root of neck, and leg.

In one bird of this species three males and two females were found; in the second, one male and one female (with adult forms of *F. bibulbosa*); in another, one male and three females (also with some adults of *F. bibulbosa*), and in the fourth, one male and three females.

The female varies in length from 20·3 to 29·4 mm.; its breadth is about 0·23 mm.

$$\text{Cobb's formula}: \frac{\text{—},\ 0{\cdot}58,\ 0{\cdot}76,\ 28{\cdot}7,\ 98{\cdot}9}{\text{—},\ 0{\cdot}62,\ 0{\cdot}62,\ 0{\cdot}69,\ 0{\cdot}31}$$

It is creamy white in colour; a long thin worm with a slightly curved tail end. The transversely striated cuticle is finely ridged, the ridging disappearing near the head end. The head end (plate VII, fig. 2) is bluntly rounded, and tapers slightly. The mouth is terminal, and is simple, bearing no papillae. The oesophagus is a straight thick-walled tube, and has no bulbous ending; the intestine commences suddenly as a broad tube full of dark granular substance, with here and there large irregularly angular masses of orange-coloured material. The position of the anus is on an average at 0·38 mm. from the posterior end; the orifice is at the summit of a low flattened papilla. No anal papillae can be made out. The body rapidly tapers beyond the anal aperture and ends in a cone-shaped portion 0·047 mm. across at its base (plate VII, fig. 3). The vulva is situated at 0·774 mm. from the head end of the worm, at the apex of a nipple-shaped papilla. The vagina courses down the worm, or may make a twist upon itself: it divides at about 1·5 mm. from its orifice into the two uterine horns, which, coiling many times on themselves, occupy almost the whole of the coelomic cavity. They end in a somewhat similar manner to that described under *F. spiralis*, except that no evidence of the existence of a 'pylorus' can be made out, and moreover, the extreme end is not bulbous.

The ovum, containing a coiled-up embryo, measures $27 \cdot 7\,\mu$ by $19 \cdot 5\,\mu$. The embryo is $112\,\mu$ long by $4 \cdot 2\,\mu$ wide.

The male is much shorter than the female, and very active when freshly introduced into normal salt solution. It is very slender, and has a well marked incurved tail. It measures 11·6 to 14·8 mm. long, and its breadth averages 0·136 mm.

$$\text{Cobb's formula: } \frac{—,\ 1 \cdot 29,\ 2 \cdot 53,\ \ 50,\ 98 \cdot 8}{—,\ 1.04,\ 1 \cdot 04,\ 1 \cdot 38,\ 0 \cdot 69}$$

The cuticle is striated and ridged as in the female. The head end (plate VII, fig. 4) is also similar to that of the female. The mouth is terminal and simple: there are no appendages.

The length of the oesophagus is 0·03 mm., there is no bulb. The anal orifice is at 0·149 mm. from the tip of the tail. The reproductive system consists of a single tube commencing thin, gradually increasing in width and occupying the greater part of the body cavity; it seems to end in connection with the bases of the spicules. The tip of the tail (plate VII, fig. 5) has four papillae, two of which are terminal so that the end appears bifid: the other two are placed dorso-ventrally to these and are much smaller. In front of these on the ventral surface are four papillae arranged in two pairs; while still further forward are two other post-anal papillae on each side; no pre-anal papillae could be made out. There are two unequal spicules, in many of the specimens extruded. (Plate VII, fig. 5, and plate VIII, fig. 1). The orifice ($16 \cdot 3\,\mu$ across) through which they protrude resembles a wide crater with sharply defined edges at the summit of a low cone. The dorsal spicule seems to widen at its base and embrace the ventral; this spicule is pointed.

The *embryos* are found in the peripheral and central blood. The length varies very much in the fresh condition from 91 to $107 \cdot 5\,\mu$: the embryo can be seen stretching itself considerably. Breadth $3 \cdot 26\,\mu$. There is no sheath (plate XIII, fig. 5). The head end is blunt and there is some differentiation into a small papillae bearing a short stumpy spine. The tail end tapers a little and ends bluntly. The contents of the body of the worm are somewhat closely granular. In the fresh condition this embryo is characterised by the possession of a very distinct oval very highly refractile globule behind the middle point of the worm, almost at the junction of the middle and posterior thirds.

In stained specimens (plate VIII, fig. 2) the embryos only measure $87 \cdot 3\,\mu$ on an average. The anterior bay in the column of cells at the head end is well marked.

The cells in these specimens appear to be loosely arranged. Four spots can sometimes be seen, but three of them are extremely variable. Sometimes one only, sometimes two, three, or four are present. One is constant—the third, and is a distinguishing feature of this embryo.

1. A small slit at a distance of 25·4 per cent. of the length of the worm.
2. A **V** which may extend across the breadth of the worm, distance 34·4.

3. A band across the worm about 4 μ wide—distance 62·2 (corresponds in position to that of the highly refractile granule seen in fresh specimens.
4. A slight lateral bay, distance 83·7.

Filaria bibulbosa. Nov. Sp.

Definitive hosts : *Cinnyris fuliginosa*.

Sites. Subcutaneously, in various positions. The worms generally occurred in pairs, male and female together. Our collection contains a single male and female in one position from one bird, and a single male from another position : this bird also contained adults. Two females were found in one bird of the same species, which also contain *F. spiralis*.

The female is a long, thin, whitish, smooth worm, both ends of which are bulbous. Its length varies from 20·7 to 22·7 mm.; its breadth is about 0·17 mm.

$$\text{Cobb's formula}: \frac{—,\ 0{\cdot}09,\ 0{\cdot}10,\ 0{\cdot}29,\ \ t}{—,\ 0{\cdot}05,\ 0{\cdot}05,\ 0{\cdot}05,\ 0{\cdot}06}$$

The cuticle is somewhat thick, smooth, not striated. There is a slight narrowing for a neck (0.13 mm. wide) separating off the bulbous head end (0·17 mm.) (plate VIII, fig 4). The mouth is terminal; no papillae nor other appendages discernible. The oesophagus is straight, has no bulb; length 0·25 mm. The anus (plate VIII, fig. 5) is terminal and central, and is surrounded by four small lips. The vulva is 0·65 mm. from the anterior end : it is situated on a low conical papilla. The vagina is directed backwards, but may coil forwards as in other filariae. The uterine horns resemble those of others previously described. The two extreme ends are bulbous; no 'pylorus' could be made out.

The male is smaller and thinner than the female, otherwise similar in appearance ; the tail end is not incurved. Length 8·6 mm. ; breadth 0·09 mm.

$$\text{Cobb's formula}: \frac{—,\ 1{\cdot}86,\ 2{\cdot}26,\ \ 50,\ \ t}{—,\ 0{\cdot}93,\ 0{,}93,\ 0{\cdot}93,\ 0{\cdot}93}$$

Width of head 0·1 : of neck 0·07 mm. The head end, mouth and oesophagus are similar to those parts in the female (plate IX, fig. 1). Posteriorly the dorsal surface is rounded off to meet the ventral surface at an angle, at which the anal orifice is situated (plate IX, fig. 2). The region round the aperture is slightly flattened. In both of the specimens in our possession, one of the spicules of this worm is extruded through the orifice. It is curved and sharply pointed ; the other appears to ensheath the former. Only one small (probably a pair) post-anal papilla can be discerned. The reproductive tube is similar to that of the other filariae.

The *embyros* are found in the blood, both peripheral and central. They have no sheath : they are capable of progression in both directions, exhibiting sinuous movements (plate XIII, fig. 6). Length 117·4 μ, breadth 4·9 μ. The body is plain

and appears structureless. The front end is bluntly rounded; and has no prepuce nor spine: the tail end tapers gradually from almost the middle of the worm to the tip of the tail.

In stained specimens (plate IX, fig. 3) the embryos set in characteristic comma-like position. The embryos measure 97·8 μ in length.

The head end does not show a baying, but simply a looseness in the cell arrangement. The following spots are always present:

1. A small central irregular clearing, at distance 22·5 per cent. of length.
2. A similar central irregularly shaped clearing, larger than the first, distance 33·5.
3. The largest spot, oval in shape, distance 60·7.
4. A tail spot, the second largest, well marked, oval, at 81·8

Filaria capsulata. Nov. Sp.

Definitive hosts: *Pyenonotus barbatus.*
Sitagra brachyptera.
Hyphantornis. Sp. incert.

Sites. In *Pyenonotus barbatus*, in the tissues between the oesophagus and spinal cord, were found three bundles which microscopically appeared to consist of a thin membranous capsule containing a worm or worms coiled up. These on dissection were found each to consist of a thin connective tissue capsule with two worms, a long one and a short one coiled up, in its interior.

In another bird of the same species were found five encysted worms between the oesophagus and spine; there was no free worm, but on dissection of one of these cysts the head of a worm was found to project about 1·0 mm. length out of the cyst. One of the cysts was very small; another large one contained a yellow coloured lightly mottled worm.

In still another bird of this species ten flattened masses were found in similar positions; they looked like bags of whitish jelly containing coiled worms. They were directly subcutaneous or on the muscle fascia with delicate fibrous tissue bands anchoring them to the tissues below, so as to permit of some movement but requiring dissection for removal. The positions in which they were found were:—one on the back of the head, three in the neck, two between the trachea and muscles of the spinal column, another at the base of the neck, one at the lower edge of the pectoral muscular mass laterally, two on the thigh.

Apparently each sack contains two worms; some of these were purposely torn across—characteristic ova and embryos issued from the ruptured uterus.

In *Sitagra brachyptera* in a single case the site was lower down the oesophagus—a cyst of yellowish colour was found between oesophagus and liver. This bird also contained embryos and adults of *F. spiralis* and *F. spiralis major.*

In the *Hypbantornis* a cyst occurred under the skin of the thigh and contained two worms.

The cyst (plate X, figs. 1 and 2) is a thin-walled delicate fibrous tissue capsule which is whitish in colour, almost transparent and closely applied to the worms which it contains. The worms are often difficult to separate entirely from the enveloping capsule. The cysts seem to contain no or extremely little fluid; each has always two worms, a male and a female. The colour of the contents varies from white to yellow, according to the colour of the contents of the intestine canal of the worms. The dimensions of the capsule vary: the largest was 5·7 by 3·4 mm., the smallest 1·8 by 1·2 mm.

The female worm, in one completely dissected-out specimen, measured 40·6 mm., breadth 0·44 mm.

$$\text{Cobb's formula: } \frac{—,\ 0{\cdot}98,\ 1{\cdot}86,\ 1{\cdot}26,\ t}{—,\ 0{\cdot}69,\ 0{\cdot}86,\ 0.75—}$$

The cuticle is thin and smooth.

The head end (plate X, fig. 3) tapers somewhat, and is bluntly rounded. The mouth is terminal; there are no papillae nor other appendages. The oesophagus is straight, has no bulb; it is 0·54 mm. long, is light in colour, and indistinctly marked off from the darker intestinal tract. In the tract of many specimens are numerous bright orange-coloured round clumps of material—which give rise to the yellow colour of the worm. These clumps are irregularly shaped—some round and others angular. The tail end (plate X, fig. 4) is bluntly rounded; the anal orifice is terminal. The vulva is situated on a small conical papilla, 0·25 mm. from the anterior end. The vagina extends directly, or after one or two forward twists, down the body of the worm for 1·6 mm. distance from the orifice, where it receives the two uterine horns. These resemble those of the *Filaria* already described. There is a well marked 'pylorus' beyond which the tube is continued for 4·48 mm., to terminate in a blunt, slightly nodular end. The ova, containing coiled-up embryos, measure 26 μ by 19·5 μ. The embryos are 81 μ long.

The male in shape and appearance resembles the female, but is much smaller. Length 4·5 mm.; breadth 0·17 mm.

$$\text{Cobb's formula: } \frac{—,\ 3{\cdot}64,\ 9{\cdot}26,\ \ 50,\ 97{\cdot}6}{—,\ 2{\cdot}64,\ 2{\cdot}64,\ 3.80,\ 2{\cdot}32}$$

The ends, both anterior (plate XI, figs. 1 and 2) and posterior (plate XI, figs. 1 and 2) are similar to those of the female. Width of head, 0.11 mm.; of tail end, 0·10 mm. Length of oesophagus, 0·39 mm. The anus is ventrally placed, a little in front of the posterior end of the worm. There are probably two spicules: one, sharply pointed, is extruded in some specimens; the other could be but very indistinctly made out.

The *embryos* occur in both central and peripheral blood. They have no sheath. Length in the fresh blood 94·5 μ, breadth 3·5 μ. The head end is slightly

tapered. The tail end tapers very gradually for the last third of the length of the worm and then at a distance of about 10 μ from the tip more rapidly, to end bluntly. The contents of the body of the worm are granular (plate XIV, fig. 7).

In stained specimens (plate X, fig. 5) the length of the embryo only averages 81·5 μ. The column of cell nuclei at the head end appears abruptly broken off—there is some looseness of the cells here also.

The 'spots': generally two are seen, sometimes only one, which is constant.

1. A narrow slit or break in the cell column; distance 32·9 per cent. of whole length.
2. Oval in shape, occupies the whole breadth of the worm; distance 58·5. This spot is constant.

Filaria shekletoni. Nov. Sp.

Definitive hosts: *Cypselus affinis.*
Hyphantornis aurantus.

Site. In *Cypselus affinis* our only two specimens, both females, were found, one lying under the pericardium along the whole length of the heart; the other in the peritoneal cavity on the upper surface of the liver. On breaking one of these, numerous embryos, similar to those found in the heart's blood, emerged.

The female is white in colour, 12·5 mm. long, 0·29 mm. broad.

$$\text{Cobb's formula}: \frac{—,\ 1{\cdot}66,\ 4{\cdot}22,\ 33.4,\ t}{—,\ 1{\cdot}44,\ 2{\cdot}11,\ 1{\cdot}88,\ 0{\cdot}55}$$

The body tapers towards the head end (plate VI, fig. 4) which is rounded and somewhat flattened dorso-ventrally. The oral orifice is terminal, and has no appendages. The oesophagus is straight, has no bulb, measures 0·5 mm. in length. The anal orifice is not quite central terminally, but is situated a little towards the ventral surface. The tail end (plate VI, fig. 5) is slightly curved and flattened. The vaginal orifice is at 0·42 mm. from the anterior end. The vagina and uterine tubes resemble those of filariae previously described. The egg measures 46·6 μ by 33 μ. The length of the embryo 315 μ.

The male is unknown.

The *embryo* in fresh blood specimens are very long, roughly measured to be about 360 μ; it has very active lashing movements, but only slowly progressive. It has no sheath; the contents are somewhat coarsely granular; the head end is rounded, has no papilla nor spine; the tail end tapers gradually to a very fine point.

In stained specimens (plate VI, fig. 2) the length averages 235 μ, breadth 6·4 μ. There is a very slight transverse striation of the somewhat thick cuticle. The head end shews no baying in the column of cells, which here ends abruptly.

Four 'spots' can be observed—all of which are constant:—

1. A narrow slit; distance 22·0 per cent. of the length of the worm from anterior end.
2. A bright oval lateral bay; distance 29·3.
3. A long portion in the middle of the worm in which the cell nuclei are few in number and stain less distinctly than the rest of the worm. Its middle point is at a distance of 61·7. This is characteristic of the worm.
4. A lateral bay, similar to and on the same side as the second; distance 84·9.

Species of Filariae, the embryos of which were found, but no adults

Filaria serpentiformis

Definitive host: *Cinnyris fuliginosa*.

F. falciformis also occurred in the blood of this bird. The embryos were found in small numbers in the blood; but many were present in the lung juice, while only a very occasional one was seen in preparations of heart's blood.

In fresh specimens (plate XIV, fig. 8) the embryos measured 436 μ; breadth 6·2 μ. They were very active, quickly coiling and uncoiling: only slightly progressive. The head end had no papilla nor spine, only a clear conical tip. Body contents granular, no distinctive spots. The tail end tapered gradually for about one-sixth of the length of the worm to a very fine point.

In stained specimens (plate XII, fig. 1) the length was 339 μ. Head end, which is slightly tapered, is round, and for a distance of about 10 μ from the tip shews no nuclear staining. A single narrow band-like or V-shaped spot only, at a distance 19·9 per cent. of total length of the worm from the anterior end can be made out. At the junction of the posterior and middle thirds is an indefinite area in which the stained nuclei are looser. The tail end consists of a single column of cells gradually diminishing in size.

This embryo resembles somewhat that of *F. shekletoni*, but it is much longer both in stained and fresh specimens and the arrangement of the 'spots' serves to distinguish them.

Filaria opobensis. Nov. Sp.

Definitive hosts: *Hyphantornis aurantus*.
Hyphantornis. Sp. incert.

Stained specimens only obtained (plate XII, fig. 2). The length of the embryo varies considerably both in specimens of blood from different birds and in

specimens from the same bird. Average 43 μ (37·5 to 61 μ). Breadth 6·6 μ. The nuclei which are very small, stain very deeply. At the head end the column of nuclei breaks into two lines to form a 'bay.' The tail end tapers in the last sixth to about one-half of its width, and then terminates in a small bulbous end. There is a very thin cuticle.

'Spots.' These can be distinctly made out, only one is constant.

1. A small irregular transverse slit, at distance 25 per cent. of length of the worm.
2. A slight lateral bay at 33·8.
3. A band across the worm at distance 60·0. This is the constant spot and a characteristic of stained specimens of this embryo.

Filaria calabarensis. Nov. Sp.

Definitive host not yet identified.

The embryos were found in central and peripheral blood. In some blood specimens (plate XII, fig. 3) these embryos occurred alone; in others, from other birds, they occurred with *F. bibulbosa* and *F. falciformis*. They were present in five birds out of nine examined. Stained specimens only available for description. Length 163 μ; breadth 4 μ. No sheath, but the thin cuticle shows very slight striation.

The anterior extremity is rounded, and there is a 'bay'-shaped opening in the column of cells, which is thus bifid. The uniform width of the worm is maintained up to the position of the third 'spot' described below, where the worm begins to taper to a very fine point.

The following 'spots' are observed:—

1. A small anterior central irregular 'spot,' distance 24·2 per cent. of length from the anterior end.
2. A shallow lateral 'bay,' at 34·6.
3. The largest and most distinct of the 'spots,' roughly diamond-shaped, at 60·8.
4. A lateral break, occurring at the same side as the second, at 82·8.

A Filaria, the adult male of which alone was found; female and embryos not observed

Filaria phoenicopteri. Nov. Sp.

Definitive host: *African Flamingo.*

The Flamingo had been skinned and cut into pieces before the time of our examination. Five male worms were found under the skin and on the muscle fascia; the examination of the available blood showed the absence of embryos.

The length of the worms averaged 13·4 mm., breadth 0·26 mm.

$$\text{Cobb's formula}: \frac{\text{—},\ 2{\cdot}67,\ 13{\cdot}3,\ 50,\ 99{\cdot}6}{\text{—},\ 1{\cdot}87,\ 2{\cdot}40,\ 2{\cdot}13,\ 1{\cdot}06}$$

The cuticle is thick, transparent, ridged, is thinner at the anterior end of the worm. The head end (plate XI, fig. 4) tapers somewhat to 0·25 mm.; there is a slight indication of a neck 0·25 mm. across. Over the position of the buccal orifice, which is terminal and central, there is a slight flattening on the edge of which are four small tubercles. The oesophagus is very long, and marked off by a constriction from the intestinal tract, which is seen to course down the worm, curving from side to side to end at the anus a little in front of the extreme tip of the tail. The tail end (plate XI, fig. 5) tapers for a considerable distance, and is incurved. The extreme end is bluntly rounded. The anal orifice is wide, placed on a slightly raised papilla; through the orifice in some specimens, the tip, in others about 50 μ length of a single sharply pointed spicule, projects.

The worms are too opaque for further details to be made out.

IV. HUMAN FILARIASIS

The species of the genus *Filaria* which are supposed to give rise to haematozoal embryos found in human blood are:—

1. *Filaria bancrofti*, COBBOLD; syn. *F. sanguinis hominis*, LEWIS; *F. nocturna*, MANSON.
2. *Filaria diurna*, MANSON.
3. *Filaria perstans*, MANSON.
4. *Filaria demarquaii*, MANSON.
5. *Filaria ozzardi*, MANSON.
6. *Filaria magalhäesi*, MANSON.
7. *Filaria loa*, GUYOT.

Filaria bancrofti

Historical. The embryo of this parasite was discovered by DEMARQUAY in 1863 in the chylous fluid from a case of dropsy of the tunica vaginalis, who came originally from Havana. WUCHERER, in 1866, found the embryos in the urine of several cases of tropical chyluria. In 1868 and following years, LEWIS, SALISBURY, CREVAUX, and COBBOLD observed the parasite in similar cases in or from Calcutta, the United States, Gaudaloupe, and Port Natal. In 1872 the history of the discovery of the life of this parasite entered a new phase, when LEWIS found that the embryos had their normal habitat in the blood of man. DA SILVA LIMA, CREVAUX, and MANSON established the identity of these blood filariae with those occurring in cases of chyluria and lymph scrotum in Brazil, the Antilles, and in China. In 1876 BANCROFT found an adult worm in an abscess in a lymphatic gland in the arm, and later four others in a hydrocele of the spermatic cord. Since then DA SILVA ARANJO, LEWIS, MANSON, and others have found adult worms in different sites. MANSON, studying the disease in China, observed a periodicity in the occurrence of the embryos of the parasite in the peripheral blood, and deduced therefrom the function of some blood sucking insect to play the part of intermediary host. In 1879 he demonstrated the life history of the parasite in the body of the mosquito, *Culex ciliaris*. As to how the parasite reached man again from the body of the mosquito several theories were advanced, until LOW, in 1900, in sectioning some of MANSON's specimens of infected mosquitoes, observed the filariae in the proboscis: which discovery naturally leads to the inference that they are introduced at the time of puncture of the skin by the mosquito.

Description. The adult *Filaria bancrofti* is a long, hair-like, transparent nematode, three or four inches in length. Males and females often are found

together; sometimes there are found several in a bunch in cyst-like dilatations of the lymphatic vessels, sometimes they inhabit the larger lymphatic vessels. The female is the larger, both in length and thickness. The length varies from 88 to 155 mm., the breadth from 0·6 to 0·7 mm. We have been unable to obtain COBB's formula for this worm. The body is plain, tapering towards the rounded head end rather abruptly to a neck, which is about one-third the width of the body; beyond which it is enlarged somewhat. The cuticle is finely striated. The mouth is terminal, simple, 4 μ in width. The tail end tapers and ends bluntly. The anus opens on the ventral surface at a distance of 0·13 mm. to 0·28 (according to the size of the specimen) from the posterior extremity, on the summit of a projection which resembles a bilobed papilla. At the extremity of the tail the cuticle presents a small depression, surrounded by two small lips. The vulva is situated at a distance of 1·26 mm. to 2·56 mm. (according to the size of the specimen) from the anterior end. The worm is ovi-viviparous. The ova measure 25 μ to 38 μ by 15 μ.

The male has a length of about 83 mm., breadth 0·407 mm. The body is cylindrical, tapering gradually from the anterior to the posterior end. The tail is vine-tendril like, the extreme end being sharply incurvating, making one or two spirals. The cuticle is delicately striated transversely. The anterior end is rounded, and not marked off by a neck from the rest of the body. The mouth is circular, simple, and terminal. The cloaca opens on the ventral surface at 0·11 mm. from the extremity. The tail end presents four pairs of pre-anal and four pairs of post-anal papillae, having a wide base. The oesophagus has a thick muscular wall, which gives it the appearance of a pharyngeal bulb: it is 0·99 mm. long, and is well marked off from the intestine. The genital tube is single. The cloaca gives exit to two unequal spicules.

The *embryos* measures from 270 to 340 μ long by 7 to 11 μ wide.

MANSON[1] describes the parasite and its movements thus:—'In fresh blood, *F. nocturna* is seen to be a minute, transparent, colourless, snake-like organism which, without materially changing its position on the slide, wriggles about in a state of great activity, constantly agitating and displacing the corpuscles in its neighbourhood. At first the movements are so active that the anatomical features of the filaria cannot be made out. In the course of a few hours the movement slows down, and then one can see that the little worm is shaped like a snake or an eel—that is to say, it is a long, slender, cylindrical organism, having one extremity abruptly rounded off, the other for about one-fifth of its entire length gradually tapering to a fine point. . . . When examined with the low power, it appears to be structureless; with a high power, a certain amount of structure can, on close scrutiny, be made out. In the first place, it can be seen that the entire animal is enclosed in an exceedingly delicate, limp, structureless sack, in which it moves backwards and forwards. This sack or "sheath"

1. Manson, *Tropical Diseases*, London, 1900; p. 485.

as it is generally called, although closely applied to the body, is considerably longer than the worm it encloses, so that that part of the sack which for the time being is not occupied is collapsed, and trails after the head or tail or both, as the case may be. It can be seen also that about the posterior part of the middle third of the parasite there is what appears to be an irregular aggregation of granular matter which, by suitable staining, can be shown to be a viscous of some sort. This organ runs for some distance along the axis of the worm. Further, if higher power be used, a closely set, very delicate transverse striation can be detected in the musculo-cutaneous layer throughout the entire length of the animal. Besides this if carefully looked for at a point about one-fifth of the entire length of the organism backwards from the head end, a shining triangular **V**-shaped patch is always visible. What may be this **V**-spot is brought out by very light staining with dilute logwood. The dye brings out yet another spot, similar to the preceding, though very much smaller; this second spot is situated a short distance from the end of the tail. The former I have designated the **V**-spot; the latter, the "tail spot." . . . Staining with logwood also shows that the body of the little animal is principally composed of a column of closely packed, exceedingly minute cells enclosed in the transversely striated musculo. cutaneous cylinder; at all events, many nuclei are thereby rendered visible. Dr. Low has recently pointed out to me that the break seen in all stained specimens in the central column of nuclei occurs at a point slightly posterior to the anterior **V**-spot. This break can only be recognized in stained specimens. When the movements of the living filaria have almost ceased, by careful focussing it can be seen that the head end is constantly being covered and uncovered by a six-tipped or hooked and very delicate prepuce; and, moreover, one can sometimes see a short fang of extreme tenuity suddenly shot out from the uncovered extreme cephalic end and as suddenly retracted.'

In the above description in all its details, our observations of the embryos occurring in cases in Nigeria completely agree; but we think that the movements of the embryos in fresh microscopical preparations previous to the stage at which the anterior tip of the 'sheath' of the worm appears to become attached to the glass, have been overlooked. If preparations be made and examined directly, it will be seen that the embryos, for a short period only, exhibit a rapidly progressive movement across the field—so rapid at first that they can only with some difficulty be traced. This movement quickly ceases, the sheath of the embryo apparently becoming attached by its tip as described.

In stained specimens in our collection we have been able to distinguish the following spots, and their positions are indicated in a manner similar to that already used in describing the embryos of avian filariae—namely in percentages of the total length from the anterior end. The measurements have been made on a number of embryos, the percentages having been found to agree very closely in each. The average total length in stained specimens was $180 \cdot 2\ \mu$.

1. An irregular transverse break, at about 21·5 per cent. of length. This is constant.
2. A V-shaped spot or a transverse irregular break at a distance of about 30 per cent. of the whole length from the anterior end. This is nearly always present.
3. Represents the central aggregation of fresh specimens: an area of varying length in which the cells are loosely arranged—distance 63. The point from which measurements were made was the middle point of this area. This is constant.
4. An irregular sometimes oval spot, often present at distance 85.
5. A small central bright spot, only occasional present at distance 91·5.

We propose here, before referring to the singular feature in the life of the embryo filaria known as 'filarial periodicity,' to describe briefly *F. diurna*.

Filaria diurna

MANSON[1] writes of this worm:—'I have twice encountered in negroes a blood worm with the same dimensions and anatomical characters, so far as these have been made out, as *F. nocturna*, but differing from this latter parasite, inasmuch as it comes into the blood during the day and disappears from it during the night. One of these patients came from Old Calabar, the other from the Congo. The periodicity observed by the parasite was thoroughly made out by prolonged observation in one of the cases. As the man was in good health at the time, and was observing ordinary habits as regards the hours of sleeping and waking, there can be little doubt that the parasite was not *F. nocturna*. Some years previously this patient had a *F. loa* in one of his eyes; it is just possible, therefore, that *F. diurna*, as I name this blood worm, is the embryonic form of the sexually mature *F. loa*. This is merely a conjecture. I have no further observations to support it; indeed, the negative results as far as finding filariae in the blood in four cases of *F. loa* which I have examined, are against it. Nothing is known about its life history or pathological significance. From recent observations I believe it to be very common (1 in 4) in certain districts on the lower Niger, where it seems to take the place among the natives that *F. perstans* holds among the Congo negroes.'

Our observations of a large number of cases of infection of what would be described as *F. diurna*, among natives from all parts of the west coast of Africa, verify the description of the blood filaria as given above by MANSON. In fact, absolutely no difference could be detected between this embryo and that of *F. nocturna*, either in fresh or in stained specimens. In stained specimens the characters and positions of the spots resemble closely those of *F. nocturna*.

1. Manson, *Tropical Diseases*. London, 1900. P. 532.

Filaria perstans.

The embryos of this worm are present in the peripheral blood both day and night. The parent forms have been described by DANIELLS, who found them in the connective tissues at the root of the mesentery, behind the abdominal aorta and beneath the pericardium. The male is smaller than the female. The body is smooth and devoid of markings.

DANIELLS[1] describes these worms, and compares their lengths and breadths with those of the adult forms of *F. bancrofti* and *F. magalhäesi*, thus :—

	F. bancrofti	*F. magalhäesi*	*F. perstans*
Length of female	95 mm.	155 mm.	70 to 80 mm.
Thickness „	0.2	0·66	0·12
Length of male	44	8	45
Thickness „	0·10	0·25	0·06

The neck is longer than in *F. bancrofti* : the mouth is very minute ; no differentiation of the alimentary canal into oesophagus and intestine could be made out. The female tail curves for the last 0·3 to 0·4 mm. Anus 0·145 mm. from the tip of tail. The tip of the tail is 'mitred.' The embryos *in utero* are blunt-tailed, not sheathed.

The male is like the female with regard to the head end. Two perfect caudal ends were found. They were very much coiled, and had one spicule and two papillae.

The embryos measure on an average 200 μ long by 4·6 μ broad : but their dimensions vary over considerable range, the embryos possessing to a remarkable degree the power to elongate and shorten. They have no sheath. The body tapers gradually for two-thirds of its length towards the tail end which is truncated and abruptly rounded. On examination of the head with the high powers of the microscope, a fang is generally observed, in constant play, protruded and retracted. No prepuce is to be made out. The movements of the embryos are extremely active, in very fresh preparations it is is almost impossible to follow them as they rapidly wriggle about between the corpuscles. Progressive movement continues for many hours.

In stained specimens the embryos of our collection on an average measure 89 μ. Four spots can be made out :—

1. A narrow irregular transverse band at distance 26·4 per cent. of total length from anterior end. Nearly always present.
2. A wider irregular transverse spot at a distance 36. Only occasionally present.
3. The largest of the spots, but not always present ; an irregular transverse area at 63·2.
4. A very inconstant central bright speck at 83·2 distance.

1. Daniells, *British Medical Journal*, 1898, vol. I, p. 1011.

In the blood of one native, a court messenger at Degema, we found on very many occasions an embryo similar to that of *F. perstans* in its movements, general shape, and appearance, but longer (average in stained specimens 151 μ). Four spots in stained specimens were made out, and were more distinctly marked than in the case of the ordinary *F. perstans*.

1. A constant narrow transverse band at a distance of 24·2 per cent. of total length from the anterior end.
2. A small lateral bay at distance 32·4. Fairly constant.
3. A distinct small area, in which the cells are loosely arranged, at distance of 61·2. This is only occasional present.
4. A small bright spot, sometimes lateral, sometimes central, at 81·2.

Filaria demarquaii

The embryo only of this worm is known. It is thus described by Manson[1] who observed it in specimens of blood from natives of St. Vincent, West Indies, in 10 out of 150 examined. 'It resembles *F. nocturna* and *F. diurna* so far as shape is concerned, but differs from them in size. I have had no opportunity of making trustworthy measurements of living specimens in suitably prepared slides, but judging from rough preparations, *F. demarquaii* appears to be rather more than half the size of *F. nocturna* and *F. diurna*. It is sharp-tailed, like these, but in addition to the size it differs from them inasmuch as it observes no periodicity, being present in the peripheral circulation both by day and by night, and, also, in not being enclosed in a sheath. Nothing is known of its life history, minute anatomy, or pathological bearings. Possibly it is the embryonic form of *F. magalbäesi*—also a tropical American blood parasite. I have recently met with apparently the same parasite in the blood of natives of St. Lucia, West Indies, where Dr. Galgey has still more recently shewn that either it, or a similar blood-worm, is very common. It is quite possible that the sharp-tailed filaria *(F. ozzardi)* of British Guiana is the same species. I have also found a minute, non-sheathed, sharp-tailed embryo filaria in the blood of natives of New Guinea, likewise closely resembling *F. demarquaii*. Whether these various embryos belong to one or to several species it is impossible to decide until the parental forms of each have been discovered and compared.'

Filaria ozzardi

A single adult female and a portion of the male found in the subperitoneal tissues in the anterior abdominal wall of an aboriginal Demerara Indian by Daniells,[2] whose blood contained nematode embryos similar to those to which

1. Manson, *Tropical Diseases*. London, 1900. P. 533.
2. Daniells, *British Medical Journal*, June 17, 1899.

MANSON originally gave the name of *F. ozzardi*, are believed to be the parent forms of these embryos. DANIELLS compares the dimensions and characters of these adults with those of *F. bancrofti* and *F. perstans*; the table we reproduce here :—

DESCRIPTION OF FILARIA OZZARDI EMBRYO

	F. bancrofti	*F. perstans*	*F. ozzardi*
	mm.	mm.	mm.
Length	85 to 90	70 to 80	81
Greatest thickness	0·20 to 0·26	0·120	0·210
Diameter of head	0·055	0·070	0·050
Diameter of neck	0·049	0·054	0·039
Distance from head—			
(1) Of vaginal outlet ...	0·710	0·600	0·710
(2) Of ovarian opening ...	0·920	?	0·850
Distance from tail of anal papilla ...	0·225	0·145	0·230
Termination of tail	Blunt, circular, not bulbous	Slightly bulbous : covered with thickened cuticle prolonged into two triangular appendages	Bulbous cuticle, not thickened

The *embryos*. We have been able to obtain only a very short and imperfect description of the embryo. OZZARD[1] and DANIELLS[2] described two embryos occurring in the blood of the aboriginal Indians of British Guiana—one a blunt-tailed worm, which has since been identified as *F. perstans;* the other, 'sharp-tailed, is about the size of *F. demarquaii* and similar in shape, but has no sheath.' No periodicity was observed in either case. 'The tail (of the sharp embryos) tapers slowly for a great length of the body to a fine and quite sharp point; the embryos arrange themselves often in figures of 8. The specimens are longer, and often in their thickest part broader than in the blunt tails. The arrangement of nuclei is clearer and more distinct, and the whole worm less deeply stained. The nuclei (of the tail end) are always arranged in single file for a considerable distance, and the terminal one has its long axis parallel to the long axis of the worm; while from this the body of the worm is continued for about 0·01 to 0·02 mm. to its termination free from nuclei. There are no nuclei at the cephalic extremity; the first ones seen are rod-shaped, with unstained spaces between, and at some little distance from the head is a gap (**V**-spot).

Filaria magalhaesi

The adult worms, which alone are known, are described by MAGALHAES[3] as having been found lying in the left ventricle of a child at Rio de Janeiro. The two

1. Ozzard, *British Guiana Med. Annual*, 1897.
2. Daniells *British Guiana Med. Annual*, 1898.
3. Magalhaes, *Rio des Cursos Theoricos e Prat da Fac. de Med. de Rio Janeiro*, No. 3, An. III, 1896.

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worms found were sexually mature. No examination of the blood had been made. The worms were cylindrical, capillary, and opalescent, white, uniform in thickness except where the body tapered towards the tail and at the club-shaped oral end; the swollen oesophagus was well marked off from the intestine. The mouth was simple, circular and unarmed, the cuticle marked with fine transverse striations. The female measured 15·5 mm. long by 0·7 thick, the male 8·3 mm. long by 0·4 mm. thick. The vulva was 2·56 mm. from the head end, at a point which divided the length of the worm in the proportion of 1 : 59. The tale of the male possessed four pairs of pre-anal and four pairs of post-anal papillae and two spicules, 0·17 mm. long. The tail made one and a half to two spirals. Nothing is known of its life history.

Filaria loa. Guyot

This worm varies from 16 to 70 mm. in length, average 30 to 40.

The female of our collection measures 50·8 mm. in length, 0·57 mm. in breadth.

$$\text{Cobb's formula}: \frac{—,\ —,\ —,\ 5{\cdot}0,\ 99{\cdot}6}{—,\ —,\ —,\ 1{\cdot}1,\ 0.39}$$

Description. The worm is of uniform thickness throughout the whole of its length, except at the head end where it sharply tapers, and at the tail where for some distance in front of its extremity, the worm gradually tapers to less than half its breadth. The cuticle bears a large number of small rounded bosses apparently irregularly arranged as also described by Manson[1] and others. The head end has the shape of a cone, with an abruptly flattened apex, at the centre of which is the small oral orifice: no buccal appendages are apparent. The specimen is too opaque for the oesophagus and its junction with the intestine to be made out. The vagina opens at a distance of 2·5 mm. from the anterior extremity. The tail end which tapers considerably, terminates in a short incurved portion (in our preserved specimen), on the concavity of which at a distance of 0·2 mm. from the extreme tip is seen the anal orifice at the summit of a low broad papilla. At the extreme end are two small fine tubercles. The ova, containing embryos, measure 35 μ by 25 μ; the embryos measure 210 μ long.

Descriptions of two male specimens are given by Manson.[1] 'Length, 25 to 30 mm., breadth, 0·30 mm. Thickness uniform except where it tapers at the head and tail. Mouth simple, no papillae nor armature. The tail end is sharply incurved and perhaps excavated ventrally; it is not spirally twisted. The tail is provided with well marked lateral alae. There are four well marked papillae on each side of the ventral surface of the tail. The three anterior papillae are pre-anal and large. They are closely approximated, stout and bulbous at the free end. The fourth is ad-anal or post-anal and is distinctly nearer the middle line and considerably

1. Manson, *Transactions of Ophthal. Soc.* London, 1895. *Case of Filaria loa*, by D. Argyll Robertson. Charles, *Sci. Mem. Medic. Officers, Army of India*; vol. vii., 1892, p. 51. Argyll Robertson, *Transactions of the Ophthalmological Society*. London, 1895. *Case of Filaria loa.*

smaller. The fifth is much smaller than the other, and is conical and sharp pointed. There are two slender, unequal spicules. The cuticle is not obviously straited but is dotted over with a number of widely scattered nearly hemispherical smooth bosses. No definite arrangement of these bosses could be made out. The large bosses are at the middle of the worm. The internal structure could not be made out.'

The *life history* of *F. loa* is quite unknown. MANSON suggests that it is the parent form of *F. diurna*.

Of the embryos, MANSON[1] says :—' The more mature embryos resemble in size and shape those of *F. nocturna* and *F. diurna*, but in consequence of the method of mounting it is impossible (speaking of the particular specimen under examination) to say if they are possessed of a sheath or not. If they are possessed of a sheath, I should say that they are practically indistinguishable from the parasites mentioned.' LEUCHART states that the embryos of *F. loa* 'are enclosed in thin egg shells, and bear a close resemblance to *F. sanguinis*, but are smaller (0·21 mm.)'

Our experience of the few cases of *F. loa* which we met with during the expedition accords with that of MANSON, in that an examination of the blood day and night did not reveal the presence of filaria embryos. We have, however, recently received a female specimen of *F. loa*, removed from the eye of a Kroo boy by Dr. A. H. HANLEY, medical officer at Opobo, Southern Nigeria. An examination of the blood showed the presence of embryos. We have counted the embryos on four slides taken at different hours of the day and night which were sent with the adult specimen.

At 10 a.m.	the blood	preparation	contained	seven	embryos
„ 3 p.m.	„	„	„	nine	„
„ 9 p.m.	„	„	„	no	„
„ 11 p.m.	„	„	„	one	„

These figures point to an infection with *F. diurna*, but the examinations being so few, in the light of the results of examinations of other cases, a very definite opinion cannot be given.

Dr. HANLEY also sent a specimen of a male *F. loa* removed also from the eye of a Kroo boy whose blood contained no embryos. We were fortunate enough to obtain at Bonny a single female of this species for our collection, and on breaking the worm across after preservation in formalin, sheathed embryos very similar to those of *F. diurna* were extruded from the broken ends of the worm. These embryos, extruded by pressure from the body of the uterus of a formalin preserved specimen, measured 208·5 μ long on an average, and have a distinct sheath, in fact, they appear similar to the embryos of *F. nocturna*. In stained specimens they measure 199·6 μ long. (It must be noticed that these embryos had been fixed in the

1. Manson, *Trans. of Ophthalm. Soc.* London, 1895. *Case of Filaria loa*, by Argyll Robertson.

body of the uterus by the formalin in which they were preserved, whereas the embryos of *F. nocturna* described and measured were fixed in blood films by absolute alcohol).

The following 'spots' were made out :—

1. An oval or diamond shape central spot, at a distance of 24 per cent. of the length of the worm from the anterior end.
2. An indistinct lateral area containing scattered nuclei—distance 37·3.
3. A longer portion of the worm which stains badly, and in which the nuclei are irregularly scattered : sometimes it is divided into two portions, anterior and posterior. Because of the bad definition of this area, its position could not be ascertained exactly.
4. A small lateral bay, at a distance of 86·2.

Filaria nocturna, diurna and perstans.

Geographical distribution. HIRSCH[1] gives an interesting account of the distribution of elephantiasis throughout the world. 'In the Eastern Hemisphere the disease is endemic in many districts : the Southern regions of the Asiatic continents and islands, such as the coast of Arabia, many parts of India, Ceylon and the Malay Archipelago, some districts of further India and the Southern and South-Eastern coasts of China. In Syria and in Japan the disease is not so common. In India elephantiasis is specially frequent along the littoral of Lower Bengal ; along the littoral swamp of the Orissa. It is found also in Pondicherry and at a few places on the Coromandel Coast ; but most of all on the Malabar coast, especially in the districts of Travancore and Cochin. In the Deccan and in upper India it occurs much less frequently, although small endemic centres exist. In Ceylon the disease is common, more especially along the coast. In the East Indies, the Lampong district of Sumatra, Banka, the Nicobars, and the Phillipines, are the regions most severely affected ; the disease is less often seen in the other islands such as Java and Amboina. It occurs also in Penang and in Cochin China.

Certain of the islands of Polynesia are among the worst regions of the globe for elephantiasis: such as the northern part of New Caledonia, the Tonga, and Fiji groups, the Samoa group, Wallis Island, the Society Islands (especially Tahiti and Raiatea), and the Gambia group. It is less common in the Marquesas and in the Hawaiian Islands. In Australia as well as in New Zealand, it is not endemic. In Africa, Réunion and Mauritius, the Seychelles, Madagascar and Nossi-Bé, the Mozambique and Zanzibar coasts, the whole coast of Upper Guinea, including the Gaboon and Cameroons country, and the Benin Coast, Gold Coast, Spice Coast and Sierra Leone, as well as the Senegambia, the disease is endemic. In parts of Tunis, Algiers, and Egypt nearest the Mediterranean, and the swampy valleys of the interior

1. Hirsch, *Handbook of Geographical and Historical Pathology*. New Sydenham Society, 1886, vol. iii, q. 712.

of Abyssinia, the disease is met with. In the upper valley of the Nile (Nubia and the neighbouring countries of the negro) elephantiasis would seem to be unknown; on the other hand there are accounts of its endemic occurrence at some places in the Greater Soudan, such as Bornou, Segu Sicorro, and Ogooué. Under the same circumstances of locality we find the disease widely endemic in the Western Hemisphere: as in the coast regions of New Granada, Venezuela, and Peru; in those parts of Brazil that are mostly tropical in character. On the coast and marshy levels of Guiana; in many islands of the West Indies such as the Barbadoes, Martinique, Guadaloupe, Trinidad, St. Vincent, and St. Bartholomew; as well as on the Gulf Coast of the Central American States of Nicaragua, Costa Rica and Panama and of Mexico.

In Europe, in Greece it is very rarely met with; it has been more frequently seen in Turkey; in the south of France also, and in Lisbon and southern Spain it would appear to be relatively common; but the patients may be in great part such as have acquired elephantiasis in the East.'

As to the demonstration of the presence of *F. nocturna* in the inhabitants of these countries, search for the adults and the microscopical examination of the blood for embryos has not yet been very extensive; but in so far as observations go, the results roughly cover the same extensive distribution as that of elephantiasis. MANSON[1] has examined blood films from many parts of the world, including Old Calabar, the Lower Niger, Dahomey, Zanzibar, Mombasa, in Africa; Madras, Cochin, Ceylon in Asia; Samoa, Fiji, the Friendly Islands in Polynesia; Georgetown, New Amsterdam, and the littoral of Demarara in British Guiana; and the islands St. Vincent, St. Kitts and Montserrat, and Trinidad among the Islands of the West Indies.

Filaria diurna. As the presence of this blood parasite is not associated with any marked pathological lesion, the determination of its geographical distribution (necessitating the microscopical demonstration of the embryos in the blood, and of their characteristic diurnal periodicity), has not been so exactly nor so extensively made. In 1900, MANSON[2] states that he has twice encountered the embryos of *F. diurna*, once in a negro from Old Calabar, and another from the Congo; and further, that from recent observations, he believes it to be very common (one in four) in certain districts on the lower Niger. This short account seems to be the whole of the present knowledge of the distribution of the *F. diurna* throughout the whole world, excepting the discovery of what, we think, must be taken as *F. diurna* in the Friendly Islands by THORPE[3]; this will be referred to again later.

Filaria perstans. For similar reasons as in the case of *F. diurna*—the absence of apparent pathological lesions and of the necessity of frequent daily microscopical

1. Manson, *Tropical Diseases*, London, 1900, p. 483.
2. Manson, *Tropical Diseases*, London, 1900, p. 532.
3. Thorpe, *British Medical Journal*, 1896, vol. ii, p. 922.

examinations of the blood—the geographical distribution of this parasite is but little known. Until recently it was believed to be confined to Africa, MANSON[1] stating that 'this parasite is very common in the blood of natives of large districts in West Africa. I have found it in natives from Old Calabar and from the basin of the Congo, both in the coast natives and in those from the interior. DANIELLS informs us that he has found it in a native of British Central Africa residing on the East side of Lake Nyassa. In many parts of the endemic districts it occurred in about half of the population. Professor FIRKET, of Liege, has confirmed this observation as regards the Congo district. Sometimes it occurs along with *F. diurna* and *F. nocturna* in the same individual. I have never found it in West Indian negros, nor in fact, in natives of any country except West Tropical Africa, and in the aborigines of Demerara. I have twice found it in Europeans who had resided in the Congo.'

OZZARD[2] and DANIELLS[3] found many cases of *F. perstans* among the aboriginal Indians of Demerara—some 130 miles up the Demerara River, and also up the Berbice River. DANIELLS also discovered the adult forms of the worms among the aborigines of British Guiana.

OBSERVATIONS ON THE DISTRIBUTION OF THE BLOOD EMBRYOS AMONG WEST AFRICAN NATIVES

We had opportunities, during the sojourn of the expedition in Nigeria, of examining the blood of natives from all parts of the West Coast of Africa, from Sierra Leone at its Western extremity, as far as the Old Calabar district at the Eastern, and from the coast inland as far as the region of the kingdom of Sokoto some 500 miles in the northern direction, and as far as Yola on the Benue river easterly. Throughout the whole of this vast area, the natives appear to be infected with *F. nocturna*, *diurna* and *perstans:* and there can be no doubt that the distribution of these parasites will prove to be much more extensive in Africa, and probably throughout the tropical world, than is at present supposed. The native Kroo boys whom we examined both day and night, generally remain in a certain place for a period varying from a few months to a number of years, usually having left their native districts after reaching manhood, returning thither at intervals. As a large number of the others examined were prisoners, these had often remained the greater part of their lives in their own countries, and had been transported to the towns at which we met them, for confinement for political, criminal, and other offences.

From our notes of cases we have made the following table, illustrating the number of cases of pure and mixed infection throughout the district mentioned above. In the table, N.D. and P. represent *F. nocturna*, *diurna*, and *perstans* respectively; N.D., N.P., D.P., represent a double infection with *F. nocturna* and *diurna*, *nocturna*

1. Manson, *Tropical Diseases*. London, 1900. P. 536.
2. Ozzard, *British Guiana Medical Annual*, 1897.
3. Daniells, *British Guiana Medical Annual*, 1898.

and *perstans*, and *diurna* and *perstans* respectively, while N.D.P. indicates the triple infection. The diagnosis of the nature of the infection is based on the examination of the blood at twelve mid-day and twelve midnight, and a case was judged to be one of *F. nocturna* or *F. diurna*, according to the presence of the larger number of filariae at one or the other time; where the numbers were close the infection was noted as a mixed *nocturna* and *diurna*, although we are aware that this may not have represented the actual state of infection, as will be seen below in the paragraph on 'periodicity.'

TABLE I.

	No. examined	No. infected with						
		N.	D.	P.	N.D.	N.P.	D.P.	N.D.P.
NATIVES OF :—								
Southern Nigeria, including the Old Calabar and Cross River districts; Bonny, Opobo and New Calabar districts; Akwete district; Brass, Wari, Sapele and Benin River districts, and the Lower Niger district extending as far as Idah ...	135	7	19	16	7	2	2	1
Northern Nigeria, including Lokoja and the regions of Sokoto, Kano, and the Benue River district	22	3	2	2	1	1	...	...
Lagos and hinterland	6	...	2	...	...	...	...	...
Gold and Ivory Coasts	4	1	...	1	...	...	...	1
Kroo Coast	40	3	1	1	2	...	1	1
Other districts, including Sierra Leone; and a few natives whose native country was not ascertained ...	18	—	1	3	—	1	—	—
TOTALS ...	225	14	25	23	10	4	3	3

The following table shows the percentage of infected natives in towns having different sanitary conditions; for example Group I contains a number of towns and villages situated chiefly near the mangrove swamp, which are usually in a deplorable filthy condition; the natives of this group were found to be infected with haematozoal embryos to the extent of 50 per cent.; whilst Group II contains comparatively clean up-country towns in the region beyond the mangrove swamp. Group III are large coast towns.

TABLE II

	Number examined	N.	D.	P.	N.D.	N.P	D.P.	N.D.P.	Number Infected
GROUP I									
Old Calabar...	35	...	8	9	1	...	1	...	19
Bonny	11	...	1	...	1	...	...	...	2
Brass	11	...	...	4	...	...	...	...	4
Okrika	4	1	2	...	1	...	...	...	4
Opobo	5	2	1	...	...	...	...	...	3
Bugama	5	...	1	1	1	...	...	...	3
Degama Town	8	1	1	...	1	1	...	...	4
New Calabar	5	2	1	...	...	...	...	...	3
Abo...	4	1	1	...	...	...	...	...	2
	88	7	16	14	5	1	1	...	44
GROUP II									
Lokoja	5	...	...	...	1	...	...	...	1
Abonnema	4	...	...	...	...	...	...	...	0
Akwete	3	...	...	...	...	...	...	...	0
Azumine	2	...	...	...	...	...	...	...	0
Obuzo	2	...	1	...	...	...	...	...	1
Abutshi	4	...	...	...	...	...	...	...	0
Idah...	2	1	...	...	...	...	...	...	1
	22	1	1	...	1	...	...	...	3
GROUP III									
Cape Coast	3	1	...	1	...	...	...	...	2
Lagos	5	...	1	...	...	...	...	...	1
S. Leone	7	...	1	...	...	...	...	...	1
Accra	1	...	...	...	...	...	...	1	1
	16	1	2	1	...	...	...	1	5

Periodicity

In the case of *F. nocturna* MANSON and others have been able on several occasions to demonstrate a 'periodicity' in the life of the blood embryo. MANSON[1] thus describes the phenomenon:—'If under ordinary conditions of health and habit, the blood of a patient be examined during the day, the parasite is *rarely* seen, *or, if it be seen only one or two specimens at most* are encountered in a slide. It would be found, however, that as evening approaches, commencing about five or six o'clock, the filariae begin to enter the peripheral circulation in gradually increasing numbers. The swarm goes on increasing until about midnight, at which time it is no unusual thing to find as many as three hundred, or even six hundred, in every drop of blood. . . . . . . After midnight the numbers begin gradually to decrease; by eight or nine o'clock in the morning the filariae have disappeared from the peripheral blood for the day. This diurnal periodicity is, under normal conditions, maintained with the utmost regularity for years. Should, however, as MACKENZIE has shown, a filarial patient be made to sleep during the day and remain awake at night, the periodicity is reversed; that is to say, the parasites come into the blood during the day and disappear from it during the night. It cannot be the sleeping state, as some have conjectured, that brings about this periodicity; for the ingress of the filariae into the peripheral blood commences three or four hours before the usual time for sleep, and the egress several hours before sleep is concluded, and this egress is not complete until several hours after the usual time of waking. . . . . . . A recent opportunity has enabled me to ascertain that, during their diurnal temporary absence from the cutaneous circulation, the filariae retire principally to the larger arteries and to the lungs, where, during the day they may be found in enormous numbers.'

To illustrate this phenomenon of periodicity we give the following table re-constructed from data given by MANSON.[2]

1. Manson, *Tropical Diseases*. London, 1900. P. 489.
2. Manson, *The Filaria Sanguinis Hominis*. London, 1883.

TABLE III

No. of Filariae per drop of finger blood

	Date	A.M.									P.M.											
		4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Case 1.	10 viii. 79	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	17	...	...	...	...
	11 ,,	...	...	...	...	0	...	...	...	...	...	0	...	...	...	...	...	16	...	...	...	...
	12 ,,	...	...	...	...	...	0	...	...	...	...	0	...	...	...	...	...	26	...	...	...	...
	13 ,,	...	...	...	...	0	...	...	...	...	...	0	...	...	...	...	...	14	...	...	...	...
	14 ,,	...	...	...	...	2	...	...	...	...	...	...	...	2	...	...	...	6	...	...	...	...
	15 ,,	...	...	...	...	...	0	...	...	...	...	0	...	...	...	...	...	4	...	...	...	...
	16 ,,	...	...	...	...	...	0	...	...	...	...	...	0	...	...	...	...	...	26	...	...	...
	17 ,,	...	...	...	...	...	2	...	...	...	...	0	...	...	...	...	...	...	12	...	...	...
Case 2.	15 vii. 79	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	13	...	...	...
	10 viii. 79	...	...	...	...	1	...	...	...	...	...	...	...	0	...	...	...	25	...	...	...	...
	11 ,,	...	...	...	2	...	...	...	...	...	...	0	...	...	...	...	...	...	...	...	...	...
	12 ,,	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	3	...	...	...	...	...
Case 3.	16 vi. 79	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	43	...	...	...	...
	17 ,,	6	...	...	...	2	...	...	...	1	...	...	...	0	...	...	...	24	...	...	...	57
	18 ,,	23	...	...	...	1	...	...	...	0	...	...	...	0	...	...	...	105	...	...	...	21
	19 ,,	18	...	...	...	0	...	...	...	0	...	...	...	0	...	1	10	29	37	...	...	...
	20 ,,	15	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	29	...	...	...	89
	21 ,,	2	...	...	...	1	...	...	...	0	...	...	...	1	...	...	...	53	...	...	...	41
	22 ,,	2	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	17	...	...	...	34
	23 ,,	5	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	24	...	...	...	43
	24 ,,	23	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	14	...	...	...	...
	25 ,,	7	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	10	...	...	...	13
	26 ,,	14	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	19	...	...	...	...
	27 ,,	11	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	10	...	...	...	...
	28 ,,	5	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	12	...	...	...	...
	29 ,,	17	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	13	...	...	...	...
	30 ,,	14	...	...	...	0	...	...	...	0	...	...	...	0	...	...	...	12	...	...	...	35
	1 vii. 79	33	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Case 4.	20 viii. 79	...	...	...	...	1	...	...	...	...	...	...	...	0	...	...	...	5	...	...	...	...
	21 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	0	...	...	...	...
	22 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	1	...	...	...	...
	23 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	0	...	...	...	...
	24 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	2	...	...	...	...
	25 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	12	...	...	...	...
	26 ,,	...	...	...	...	0	...	...	...	...	...	...	...	0	...	...	...	2	...	...	...	...
	27 ,,	...	...	...	...	1	...	...	...	...	...	...	...	0	...	...	...	0	...	...	...	...

Table IV, showing experimental inversion of filarial periodicity constructed from Manson's Chart.—No. of filariae in preparation under 1 x 1½ inch cover glass.

Date	A.M.						P.M.					
	2	4	6	8	10	12	2	4	6	8	10	12
9. xii. 79	...	...	...	...	...	...	...	...	0	...	**58**	...
10 "	...	...	**63**	...	0	0	...	...	0	...	**62**	...
11 "	...	...	**96**	...	6	0	...	0	10	...	**30**	...
12 "	...	...	**155**	...	8	0	...	0	8	...	**88**	...
13 "	...	...	**70**	...	6	0	...	0	8	...	**26**	...
14 "	...	...	**48**	...	7	0	...	...	0	...	38	**115**
15* "	...	...	**185**	...	12	6	...	...	0	...	8	**12**
16* "	...	...	**52**	...	**46**	8	...	...	6	...	8	**28**
17* "	...	**38**	8	...	**70**	14	...	...	6	...	8	6
18* "	...	8	48	...	**38**	**60**	...	...	5	...	5	8
19* "	...	5	**74**	...	**76**	**50**	...	**88**	5	...	4	6
20* "	...	8	**38**	...	**62**	**52**	...	...	10	..	2	8
21* "	...	15	**42**	...	**36**	**34**	...	...	11	...	10	11
22* "	...	11	28	...	**60**	46	...	...	10	...	6	6
23* "	...	15	**54**	...	48	**95**	...	...	24	...	8	4
24* "	...	12	21	...	68	**86**	...	...	18	...	8	8
25 "	...	9	15	...	...	...	...	...	...	...	...	...

We have had to construct this table from a chart in which the number of filariae were recorded by dots placed between horizontal lines, each representing ten filariae, and thus the numbers may not be exactly correct (within one to five units), owing to the difficulty of gauging the number represented by a dot placed between such lines.

On days marked thus * the sleeping hours were from five a.m. to five p.m. On other days from six p.m. to six a.m. On December 14 the patient was not allowed to sleep. The experiment had been previously made by Mackenzie[1] with similar results.

1. Mackenzie, *Trans. Path. Soc. of London*, vol. xxxiii, p. 400.

The following tables illustrating the periodicity of *F. nocturna* have been constructed from our own notes of a number of cases among West African natives. Three specimens were made from each case every three hours. Sufficient blood was taken, to form as nearly as possible a complete film under a cover glass three-quarter inch square, and the specimens were examined in the fresh condition. Throughout the following tables the maximum number of filariae in three slides is indicated by a larger type of figure.

TABLE V

Name	Date	Number of Filariae in three slides at							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Oparobo ...	11. vii. 00 ...	20	0	0	0	0	10	**48**	26
2. Deafman ...	12. vii. 00 ...	0	2	0	0	0	0	1	**3**
3. James ...	12. vii. 00 ...	7	0	1	0	0	0	**11**	9
4. Abraham ...	27. viii. 00 ...	0	1	0	1	0	0	**4**	2
5. Onye mensoh	27. viii. 00 ...	18	2	0	0	0	25	45	**56**
6. Sumanu ...	27. viii. 00 ...	7	0	0	0	0	17	**21**	9
7. Osadebe ...	27. viii. 00 ...	28	2	1	0	0	35	34	**50**
8. Eyamah ...	27. viii. 00 ...	2	0	0	0	0	3	7	**19**

As to the periodicity of *F. diurna*, Manson[1] says simply that the parasites come into the blood during the day and disappear from it during the night; and, the periodicity observed by the parasite was thoroughly made out by prolonged observation in one of the cases. Actual records we have not been able to find.

From our own collection of records of cases we have constructed the following table illustrating the periodicity of *F. diurna* : the figures represent the number of embryos in three specimens of blood under a three-quarter inch square cover glass.

1. Manson, *Tropical Diseases*, London, 1900. P. 532.

TABLE VI

Name	Date	No. of Filariae in three specimens at							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Robert ...	12. vii. 00 ...	0	8	**84**	**58**	**66**	47	0	0
2. Adeyemi ...	27. viii. 00 ...	0	3	8	**48**	7	3	0	0
3. Obudu ...	27. viii. 00 ...	0	10	21	**32**	...	8	1	0
4. Garuba ...	27. viii. 00 ...	0	1	27	**52**	38	9	0	0
5. Apanituen ...	20. vii. 00 ...	1	3	7	**8**	3	0	0	0

It has not been easy to pick out from our records a fair number of cases either of *F. nocturna* or *diurna* which may be said to be absolutely typical ; thus only eight cases of *F. nocturna* and only five of *diurna* could be found. We propose to call a case typical when the maximum number of embryos are present in the blood at mid-day or midnight as the case may be, or about those hours and when twelve hours later they are absent from peripheral blood.

The majority of the cases which we encountered on the West African coast were then atypical, in that, embryos were never absent from peripheral blood, or the maximum did not occur at mid-day and midnight or thereabouts according to the species. Among the former cases there were many shewing decided periodicity and among the latter, the hour at which the maximum number was present, varied considerably. In some cases two maxima during the twenty-four hours were indicated. Table VII shews a few cases in which though a decided periodicity is to be noted, embryos are never absent from peripheral blood.

TABLE VII

Name	Date	Number of Embryos in three specimens at							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Davis ...	12. vii. 00 ...	12	61	425	**478**	197	252	12	9
2. Ajaca ...	27. viii. 00 ...	9	**20**	10	5	...	3	**35**	18
3. Arrigwe ...	3. vii. 00 ...	7	6	16	**58**	**58**	45	15	4
4. Ijululockia ...	20. vii. 00 ...	15	18	56	**61**	25	1	1	3

Table VIII gives a number of cases in which the maximum number did not occur at mid-day nor midnight.

TABLE VIII

Name	Date	Number of Embryos in three specimens at: A.M. 3	6	9	12	P.M. 3	6	9	12
Etta ...	3. vii. 00 ...	12	3	8	20	**46**	25	21	21
Jumbo ...	3. vii. 00 ...	0	1	2	4	**18**	4	0	0
Efion ...	12. vi. 00 ...	0	11	7	5	**16**	6	0	0
Greenslade ...	12. vii. 00 ...	0	1	28	54	76	**81**	15	0
Glasgow ...	12. vii. 00 ...	4	**35**	...	9	20	**49**	3	0
Joe ...	12. vii. 00 ...	3	0	0	0	0	**4**	**12**	6
Oparobo ...	12. vi. 00 ...	20	0	0	0	0	10	**48**	26
Kelba ...	12 vii. 00 ...	2	1	0	3	5	8	**15**	7
James ...	12. vii. 00 ...	7	0	1	0	0	0	**11**	9
Abraham ...	27. viii. 00 ...	0	1	0	1	0	0	**4**	2
Sumana ...	27. viii. 00 ...	7	0	0	0	0	17	**21**	9
Ajaca ...	27. viii. 00 ...	9	20	10	5	...	3	**35**	18
Emordi...	27. viii. 00 ...	**54**	5	0	3	3	15	22	44
Arrigwe ...	12. vi. 00 ...	14	**25**	5	**27**	8	18	5	8
Mark ...	12. vi. 00 ...	2	**66**	28	22	...	18	2	1
Okohorsfall ...	20. vii. 00 ...	42	43	**54**	26	25	29	5	9
Deauma ...	20. vii. 00 ...	4	60	**130**	68	47	1	1	0
Robert ...	12. vii, 00 ...	0	8	**84**	58	66	47	0	0
Etim ...	12. vi. 00 ...	3	6	**16**	6	8	0	0	0

THORPE[1] examined a number of natives of the Friendly Islands; but his results, as recorded in the article referred to, do not permit very definite conclusions. We have however reproduced them in the following tables copied from his article.

1. Thorpe, *British Medical Journal*, 1896, vol. ii, p. 922

TABLE IX

	Natives of Tongatabu			Natives of Normuka			Natives of Lifuka			Natives of Vavau			Totals		
	No. exam.	No. infect.	Per-centage	No. exam.	No. infect.	Per-centage	No. exam.	No. infect.	Per-centage	No. exam.	No. infect.	Per-centage	No. exam.	No. infect.	Per-centage
Males examined :															
Day and night ...	7	3	...	25	12	...	23	12	...	...	...	...	55	27	49
Day only... ...	3	0	...	3	0	...	2	1	...	...	...	...	8	1	...
Night only ...	31	11	...	9	3	...	6	5	...	14	4	...	60	23	...
	41	14	34·1	37	15	40·5	31	18	57	14	4	28·6	123	51	41·47
Females examined :															
Day and night ...	4	3	...	26	7	...	11	3	...	...	...	...	41	13	31·7
Day only... ...	3	0	...	6	0	...	...	...	...	...	...	...	9	0	...
Night only ...	17	2	...	11	1	...	7	2	...	6	0	...	41	5	...
	24	5	20·8	43	8	18·6	18	5	28	6	0	...	91	18	19.9
Totals for whole population ...	65	19	29·23	80	23	28.75	49	23	47	20	4	20	214	69	32·24

TABLE X

Name	A.M.			P.M.				
	9½	10	12	2½	5½	6½	8½	10
Tubon	...	21	9	16	17	...	30	...
Saen	22	...	27	...	...	16	...	...
Kesaia	...	80	...	...	...	...	...	56

The numbers represent the number of embryos in a drop of blood under a seven-eighths inch circular cover glass.

Referring to the day and night examinations, Thorpe says that no periodicity was observed: that the embryos resembled *F. nocturna;* they had a sheath, and exhibited the characteristic preputial collar and V-spots. He gives a number of measurements which correspond to those of *F. nocturna*, except that the worm appears to be a little smaller than that of China and India. In ninety-six cases examined, all

except two had an equal number of embryos in the blood both day and night: of the two exceptions, one showed a single parasite at night, none in the daytime; the other a single parasite at the day examination, none at night.

In spite of the small number of examinations and of their incompleteness, it is certainly evident from the above figures that the parasite does not agree in any way in its occurrence in the peripheral blood with either *F. diurna* or *F. nocturna.* It must however be noted that THORPE describes the Friendly Islands as 'a hot-bed of elephantiasis.' This point will be referred to later.

The following tables illustrate how the occurrence of embryos in the blood varies from day to day and week to week in the same cases. It must be here remarked, that the habits of the men whose blood was frequently examined for the purpose of the construction of these tables, were marked by extreme regularity. They were government prisoners, kept in the government prison at Bonny. The men rose at five o'clock, were fed at eleven o'clock mid-day, and were locked up in their cells about eight o'clock; from five till eleven and from twelve till six they were at work.

In every case three drops of blood were examined under a three-quarter inch square cover glass.

TABLE XI

NAME	DATE	NUMBER OF FILARIAE IN THREE BLOOD SPECIMENS AT							
		A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Arrigwe ...	12. vi. 00 ...	14	25	5	27	8	18	5	8
	3. vii. 00 ...	7	6	16	58	58	45	15	4
	7 ,, ...	...	...	...	18	...	...	...	...
	9 ,, ...	...	...	...	...	38	...	...	...
	10 ,, ...	...	...	...	...	43	...	...	...
	11 ,, ...	...	...	...	...	89	...	...	...
	12 ,, ...	...	...	...	...	13	...	...	...
	13 ,, ...	...	...	...	...	32	...	...	...
	14 ,, ...	...	...	...	...	53	...	...	...
2. Etta... ...	12. vi. 00 ...	46	50	27	26	24	6	5	17
	3. vii. 00 ...	12	3	8	20	46	25	21	21
	7 ,, ...	...	...	...	...	17	...	...	...
	9 ,, ...	...	...	...	...	4	...	...	...
	10 ,, ...	...	...	...	...	10	...	...	...
	11 ,, ...	...	...	...	...	17	...	...	...
	12 ,, ...	...	...	...	...	14	...	...	...
	13 ,, ...	...	...	...	...	9	...	...	...
	14 ,, ...	...	...	...	...	9	...	...	...
3. Efion ...	12 vi. 00 ...	0	11	7	5	16	6	0	0
	3. vii. 00 ...	0	4	16	15	10	1	0	0
	9 ,, ...	...	...	...	...	9	...	...	...
	10 ,, ...	...	...	...	...	13	...	...	...
	11 ,, ...	...	...	...	...	36	...	...	...
	12 ,, ...	...	...	...	...	52	...	...	...
	13 ,, ...	...	...	...	...	18	...	...	...
	14 ,, ...	...	...	...	...	56	...	...	...
4. Jumbo ...	12. vi. 00 ...	1	4	8	1	4	0	0	0
	3. vii. 00 ...	0	4	16	15	10	1	0	0
	9 ,, ...	...	...	...	...	10	...	...	...
	10 ,, ...	...	...	...	...	0	...	...	...
	11 ,, ...	...	...	...	...	2	...	...	...
	12 ,, ...	...	...	...	...	2	...	...	...
	13 ,, ...	...	...	...	...	7	...	...	...
	14 ,, ...	...	...	...	...	6	...	...	...
5. Etim ...	12. vi. 00 ...	3	6	16	6	8	0	0	0
	3. vii. 00 ...	0	0	17	9	8	5	0	0
	9 ,, ...	...	...	...	...	26	...	...	...
	10 ,, ...	...	...	...	...	6	...	...	...
	11 ,, ...	...	...	...	...	23	...	...	...
	12 ,, ...	...	...	...	...	14	...	...	...
	13 ,, ...	...	...	...	...	6	...	...	...
	14 ,, ...	...	...	...	...	10	...	...	...

In this table the point to be noticed is that a considerable amount of variety occurs in the way in which embryos present themselves in the peripheral circulation in those cases in which the type (*F. nocturna* or *F. diurna*) is not strictly adhered to.

For instance, in Cases 3, 4, and 5, the numbers at each examination are as near as would be expected; but in Cases 1 and 2 the variations from day to day are considerable.

In the above tables, III to XI inclusive, a further feature is to be observed, namely, the variety in the severity of the filarial infection: thus, taking the numbers of embryos at the period when they reach a maximum in peripheral blood, it is seen that they are included between 3 and 480 in three specimens, or 1 and 160 per specimen of blood. It surely follows, then, that in some natives even when they are at their maximum number, in the peripheral blood, they may still be too few, *in toto*, to be observed in a single preparation of blood. Consequently, many more natives must be habitats for filariae than is supposed from the observation of peripheral blood in the usual way. When treating of *F. perstans* (see Table XII, Case 4), it will be seen that in some sixty preparations of the blood of one case, one filaria only was observed (possibly the infection with *F. perstans*, or the maturation of the parasite, may have occurred during the month under which the case was under observation). MANSON's[1] figures shew the same features in the cases of undoubted *F. nocturna* infection; but few of these figures, however, give the number of embryos per drop of blood when the largest number would have been present in peripheral blood, namely, twelve midnight: most of the specimens were made not later than ten p.m.: the figures range between 1 and 105. These facts must be taken to give some indication of the severity of the infection, of the number of adult females in the organism: since the results of observations extended over a long period—a month or more—shew no decided periods of increased fertility. But it surely must not be inferred from the relative numbers of embryos in the two extreme cases that the number of adult females in one case is a hundred or more times as many as in another, although it is difficult, at the present stage of our knowledge, to understand why such an inference should not be drawn. Referring to this subject, MANSON[2] is reported to have said—'If anyone is foolhardy enough to submit to be bitten by filariated mosquitoes, and if subsequently no young filariae be found in the blood, it must not be concluded from this that a mosquito bite is not the medium of infection. My belief is that before embryos can be found in the blood by ordinary miscroscopic observation large numbers of parent filariae must be present in the lymphatics. In many cases we know that hundreds of parent filariae are present. Thus in one case only two or three embryo filariae are found in each drop of blood; in other instances as many as 600 or more are found in a drop implying the presence of 300 times as many parental worms.' Although as above stated we do not at present understand why such an inference cannot be deduced, it is evidently not justifiable to make such an inference, judging from the number of infected inhabitants and the extent of their infection. We have not been

1. Manson, *The Filaria Sanguinis hominis.* London, 1883.
2. Manson, *Brit. Med. Journal*, Sept. 1. 1900. P. 536

able to find any record of a case or cases in which the embryos were regularly counted for a period shortly before death and in which, *post-mortem*, adults were found.

In the examination for malarial parasites[1] of blood specimens from a large number of native children of all ages up to about 18 years, we encountered a single filarial embryo only, in one case (specimens taken during the day were examined only)—aged 11 years, out of 390 cases. In view of the number of adults infected with *F. diurna* in the same districts, this is remarkable and further tends to support the idea that, the extent of infection increases during the period of childhood, until, when adult age is reached, there are a sufficient number of mature female filariae in the body to give an observable number of embryos in peripheral blood during the usual examination for microscopical purposes.

Under any other circumstances, it seems to us, there would be no chance of an escape from the continued and renewed infection of every individual. In a certain district, were such a number of embryos observable in the blood of every child—or even of a similar percentage of children, as is presented by the adults, every mosquito of the species capable of acting as intermediary hosts would become infected and in consequence every man, woman and child in that district would become infected to such an extent as to exhibit embryos in the peripheral blood. It thus seems that in this way nature has placed a limit to the prevalence of this infection.

Filaria perstans

But little need be said of the periodicity of this worm, which persists in the peripheral blood throughout the whole of the day. The following table illustrates the phenomenon.

1. *Report of the Liverpool Malaria Expedition to Nigeria.* Liverpool, 1901, part i, p. 11 *et seq.*

TABLE XII

Name	Date	A.M.				P.M.			
		3	6	9	12	3	6	9	12
1. Ekpeyon ...	12. vi. 00 ...	0	7	1	1	0	2	4	3
	3. vii. 00 ...	2	2	5	3	5	5	1	4
	9 „ ...	...	...	...	...	6	...	...	...
	10 „ ...	...	...	...	...	3	...	...	...
	11 „ ...	...	...	...	...	10	...	...	...
	12 „ ...	...	...	...	...	3	...	...	...
	13 „ ...	...	...	...	...	2	...	...	...
	14 „ ...	...	...	...	...	2	...	...	...
2. Etim ...	12. vi. 00 ...	1	1	0	0	0	0	4	1
	3. vii. 00 ...	2	2	1	1	0	4	4	1
	9 „ ...	...	...	...	...	1	...	...	...
	10 „ ...	...	...	...	...	2	...	...	...
	11 „ ...	...	...	...	...	2	...	...	...
	12 „ ...	...	...	...	...	1	...	...	...
	13 „ ...	...	...	...	...	0	...	...	...
	14 „ ...	...	...	...	...	10	...	...	...
3. Joe	12. vi. 00 ...	3	0	2	2	0	4	0	1
4. Efion ...	12 „ ...	0	0	0	0	0	0	0	0
	3. vii. 00 ...	0	0	0	0	0	0	0	0
	10 „ ...	...	...	...	...	0	...	...	...
	14 „ ...	...	...	...	...	1	...	...	...
5. Ijuluockia ...	19 „ ...	0	1	9	3	7	7	3	2
6. Demai ...	19 „ ...	1	0	2	2	0	0	1	0
7. Malam ...	27. viii. 00 ...	0	2	2	7	2	1	4	2
8. Obudu ...	27 „ ...	3	2	0	0	...	0	1	2
9. Sumanu ...	27 „ ...	1	1	0	0	0	0	4	1

The only striking feature which this table presents is the smallness of the number of *F. perstans* embryos; the greatest number we ever observed, was thirteen in three specimens under three-quarter inch square cover glasses; that is, approximately 2 to 3 c.mm. of blood.

THE INTERMEDIARY HOST OF FILARIA NOCTURNA: ITS DEVELOPMENT.

The phenomenon of the periodicity of *F. nocturna* led MANSON to induce the further development of the parasite in a blood-sucking insect of nocturnal habits. In 1878 MANSON demonstrated developmental changes in the embryos after ingestion by the mosquito, since when the whole of the life history in the intermediary host has been observed by BANCROFT, JAMES, SONSINO, Low and ourselves in *Culex pipiens*, *C. ciliaris*, *C. fatigans*, *Anopheles costalis*, *Anopheles rossii*, BANCROFT has shewn that *C. notoscriptus* (SKUSE), *C. amuli rostris* (SKUSE), *C. hispidosus* (SKUSE) *C. vigilax* (SKUSE), *C. nigrothorax* (MACQUART), *C. procax* (SKUSE) and *Anopheles musivus* (SKUSE) do not serve as intermediary hosts.

MANSON[1] gives the details of the various stages of the metamorphosis of *F. nocturna* embryos in *C. pipiens*.

First Stage. Transverse striation becomes well marked as if from a general longitudinally shrinking of the embryo; oral pouting vigorous. In about one hour the embryo casts its sheath; and then shows active locomotive movements. In from twelve to eighteen hours many have bored through the stomach wall of the mosquito and have reached the muscles of the thorax. Some die in the stomach. In the thorax, the striation disappears and movement ceases: the body becomes thicker and an illdefined cloudiness appears in the interior.

Second Stage. The body thickens, and there is a faint indication of a mouth; this stage requires two to three days for completion.

Third Stage. The anus appears, and cells are seen in the body; the mouth becomes open, and gradually four large fleshy lips are fashioned. The anus appears in front of the tail as a break or hole in the cuticle, from which granular matter exudes. The line of the cells, which are now visible in the previously apparently homogenous body, does not terminate at the anus but in advance of this, in some large prominent cells. The cells later become differentiated into an alimentary layer, and a tegumentary layer with a cavity between. The larva now measures $\frac{1}{800}$ to $\frac{1}{80}$ inch long (0·25 to 0·3 mm.) and $\frac{1}{850}$ to $\frac{1}{500}$ inch broad (0·048 to 0·45 mm.) There is considerable diversity in size and shape. The mouth is wide open; the tail is large and sickle shaped, and the cells of the body usually dip into it. The alimentary canal runs from mouth to anus. Motion is entirely suspended.

Fourth Stage. Growth is rapid: length $\frac{1}{70}$ to $\frac{1}{50}$ inch (0·35 to 0·5 mm.) The body retracts from the tail, which becomes a mere integumental appendage.

1. Manson, *Transactions of the Linnean Soc.*, 1884. P. 367

Fifth Stage. When the body has attained its maximum thickness, lengthening and thinning begin at the head end. The mouth inclines to purse up. The anterior and posterior ends may elongate simultaneously; more generally the process occurs throughout the whole length of the body of the larva. When the mouth closes, as it does later, all or nearly all trace of viscera and all traces of cells vanish. About the seventh day the body assumes a fibrous and very transparent appearance. Before this stage there can be made out a fully moveable alimentary canal, pharynx and oesophagus. Slight movements commence at the neck of the animal and extend downwards. MANSON thinks that about this stage a general ecdysis occurs, and the sickle shaped tail is cast off: a new skin can be seen covering the tail end, inside the sickle. Large cells appear at the end of the tail and form three or four papillae which characterise the larva at the end of this and during the next stage.

The worm has now reached a length of $\frac{1}{16}$ inch in length (1·5 mm.), its breadth has decreased to about one-half. The anterior end tapers and is abruptly rounded off; the posterior end also tapers slightly from the anus backward and is covered by the papillae just mentioned.

Sixth Stage. Movements become more active. The mouth is pursed up into a cone with lips firmly approximated; minute horny papillae are present. . . . The worm measures $\frac{1}{16}$ by $\frac{1}{850}$ inch (1·5 mm. by 0·03 mm.)

Up to 1900, this was supposed to be the complete development of the filaria in the mosquito, and at this stage it was conjectured that, on the death of the mosquito on the surface of the water, the young filaria escaped from the insect and swam about until it was taken up by man in drinking water.

In 1900 Low[1] in sectioning a number of filariated mosquitoes discovered a worm in the proboscis. He thus describes the transformation into the seventh stage:—'When the filariae have reached their highest stage of development in the thoracic muscles, they leave that tissue and travel forward in the direction of the head of the mosquito and pass into the loose cellular tissue which abounds in the prothorax near the salivary glands. Some struggle between the thorax and abdomen or within the abdomen itself. They then pass into the neck, enter the lower part of the head and coil themselves up in the loose connective tissue immediately below the cephalic ganglion and salivary sack. They pass into the proboscis by making an independent passage through the base of the labrum and pushing forward along the proboscis between the labrum and hypopharynx amongst the stilettes. Here they are found stretched along the length of the proboscis, head foremost. Two worms nearly always live together in the proboscis.'

JAMES[2] apparently was working at this subject at about the same time, and writes in an article, dated September, a description of the worms as seen in *Anopheles*

1. Low, *British Medical Journal*, 1900, June 16
2. James, *British Medical Journal*, 1900, vol. ii, Sept. 1, p. 535.

rossii on the seventeenth and eighteenth days of cultivation. 'The young filariae are found in the tissues of the thorax, in those of the head and neck, and in fewer numbers in those of the abdomen. The tissues of the head are examined by cutting through the neck. . . . . By carefully dissecting with needles the tissues of the head, and separating the parts of the proboscis, two or three filariae will almost invariably be found in this situation, and I have lately on two occasions found a filaria lying stretched out lengthwise partly within the tissues of the labrum of the proboscis, the remainder of its body being curled up in the tissues of the head. Without dissecting up the tissues of the labrum these filariae could be plainly seen with a $\frac{1}{6}$ inch objective through its fairly transparent tissue indulging in sinuous undulatory movements, and a very little manipulation with the needles sufficed to free the filariae when their movements changed from the snake-like undulatory character to the vigorous purposeless lashing and twisting which are characteristic of the final stage of the metamorphosis of the parasites in the mosquito. In the diagram I have shown the appearance of the filaria as it lies partly within the labrum. . . . . . . The young filariae in the final stage are from $\frac{1}{14}$ to $\frac{1}{16}$ of an inch in length, and $\frac{1}{800}$ inch in greatest breadth. It tapers towards the head and tail; the latter has three projections which can be spread out or drawn closely together in the animal's movements. The head end is rounded, and the mouth which is very extensile can be pushed out to form a little cone-like projection which sways from side to side, and is drawn in and pushed out as if searching for food. The filariae have an alimentary canal which at a somewhat earlier stage can be seen to be very freely moveable within the animal's body, and to be of varying shape in different parts of its course. Near the anus it is wide, and then narrows gradually to open at a short distance from the tail. On each side of the alimentary canal near the head, and again at a point about the middle of the body, the protoplasm is differentiated into other organs—probably reproductive.'

On August 4, 1900, as a result of some experiments which we had been carrying on as opportunities presented, during the time we were in Southern Nigeria, we were able to cable home that a living filaria had been found in the proboscis of *Anopheles costalis*. Previous to this several attempts had been made by us to cultivate both *F. nocturna* and *F. diurna* in mosquitoes of both genera, *Culex* and *Anopheles*, but without success. In this experiment with *F. nocturna* we were however successful. We had on several occasions previously noticed that large filariae were to be seen in the head of mosquitoes. The mosquitoes were fed on two occasions on blood containing embryos—July 18 and July 20, and in order to keep them alive for a period they had occasionally been fed on blood containing no embryos. On August 4 only five *Anopheles* of the batch remained, two of them being dead on the water. One of these on dissection proved negative. In the other, in the proboscis and near a trachea in the labium of that organ was a long thin filaria. In the thorax of this

mosquito a similar worm of the same size was found. A living mosquito was then taken, quickly killed by chloroform, and without any dissection whatever the thorax was pierced by a needle, and the finger nail placed on the tip of the proboscis. The parts were then gently drawn apart, the labium and palpi being thus separated from the stylets. A very active filaria was then seen to curl itself out from the neighbourhood of the trachea of the labium. This lived for about an hour in normal saline, coiling and uncoiling itself. The other two *Anopheles* died during the following night and on dissection proved to be negative.

In this stage, *the seventh*, the worm according to our specimen, is about 1·006 mm. long and 0·025 mm. broad. It tapers slightly to each end. At the anterior end, which is rounded off, the cuticle is thickened in places to form a few very small papillae disposed around the oral orifice, which is terminal. The posterior end, which is also rounded off, is provided with four papillae which are almost at right angles to the axis of the body of the worm. The position of the anal orifice cannot be definitely decided. The alimentary canal can be seen to run straight down the worm and shows no differentiation as far as we have been able to ascertain in oesophagus and intestine. Besides the alimentary tube in parts two other tubes can be seen which are for the most part straight, but at one or two points seem to twist round the intestine. Towards the head end at a distance of 0·14 mm. from the anterior end, there is an indication of the presence of an orifice towards which the reproductive tube is seen to bend.

Filariae in Anopheles costalis

According to our notes 281 *Anopheles* were examined for filariae—sixteen of these (5·7 per cent.) were found to contain the worms. The following are the details of the examinations :—

1. Two large filariae.
2. Eight to ten among thoracic muscles.
3. Several young larvae.
4. About ten large forms in thorax.
5. Several large forms among the thoracic muscles.
6. A single large filaria.
7. A small larva dissected out from the head.
8. Eight small larvae in thorax.
9. A few young forms in thorax.
10. Four young larvae.
11. Ten larvae found.
12. Fourteen larvae in thorax.
13. Several large larvae.
14. A single larva.
15. A single large larva found at the base of the proboscis.
16. Ten filariae in thorax.

The Anatomy of the Mouth parts of the Female Anopheles Costalis

The discovery of the final stage of the metamorphosis of the larval stage of *F. nocturna* in sections of the proboscis of mosquitoes, by Low,[1] in 1900; the observations of JAMES[2] as to the way in which the matured larvae tend to travel through the tissues of the head and neck into the proboscis; the work of GRASSI[3] on *F. immitis* in the dog, and in *Anopheles claviger*, in which the larvae were found in the labium; and our own researches in West Africa on the life history of *F. nocturna* in the body of *Anopheles costalis*; make a knowledge of the minute anatomy of the proboscis of the mosquito requisite for an exact understanding of how the larva leaves the insect and is transmitted to man.

As far as we have been able to ascertain very little has been done by entomologists in this country in the investigation of the histology of the proboscis of the mosquito; it has chiefly been examined from a morphological point of view, as of importance in the classification of species.

METHODS OF INVESTIGATION EMPLOYED

1. Examination of the proboscis of living insects.

2. Dissection of specimens in the fresh condition; and the examination of the organ hardened in alcohol and cleared in oil of cloves, and mounted as a whole or in parts in Canada Balsam.

3. By sections. Mosquitoes were killed by chloroform vapour; hardened in absolute alcohol for one or two hours, and embedded in paraffin. Serial sections (6 to 10 μ thick) of the proboscis and head were cut in three directions. Thin paraffin sections of the proboscis are with difficulty fixed to a slide by the ordinary methods of laboratory practice; the chitinous skeleton tending to break away from the delicate tissues which it encloses, in the processes of the manipulation of paraffin sections, so that minute anatomical relations become disturbed and obscured.

By the use of a slight modification of OBREGIA'S[4] method for fixing sections cut in paraffin, we were able to secure excellent results. A mixture of two parts of commercial liquid glucose and one part of a thick syrup of pure dextrin* is spread in a thin layer on to the glass slide by means of a glass rod. The serial paraffin sections as they are cut are laid directly on this layer on the slides, which are then placed in an incubator at about 40° c. for some hours, until the glucose mixture has dried hard. The paraffin is then removed by means of xylol and the slide with the sections is passed through absolute alcohol. A solution of photoxylin is then poured over the slide, so as to form a thin film over the sections; this layer of photoxylin is

1. Low, *British Medical Journal*, 1900. June 16.
2. James, *British Medical Journal*, 1900. Vol. ii, Sept. 1, p. 535.
3. Grassi, *British Medical Journal*, Nov. 3, 1900.
4. Obregia, *Neurologisches Centralblatt*, 1890. Gulland, *Journal of Pathology*, Feb. 1893, p. 391.

* 16 oz. dextrin; 17½ oz. water; 15 grains thymol.

allowed to set until the edges of the film begin to crinkle. On placing the slide in water, the film comes away with the sections which are now ready for staining *in situ* in the film. Carbol-xylol must be used for clearing after dehydration.

Most of our observations were made on *Anopheles costalis*, but a few specimens of *Anopheles maculipennis* and of different species of *Culex* have also been examined by dissection.

External anatomy of mouth parts

The term 'proboscis' is used to designate such of the mouth parts of *Diptera*, which taken together form their more or less flexible, shorter or longer, sucking apparatus. In the Culicidae the proboscis is a long slender organ arising from the lower projecting portion of the front of the head, beneath the clypeus or face. On its ventral surface it is continuous with the under surface of the head—the gulo-mental region. Its upper surface is sharply marked off from the clypeus by a deep groove. In a transverse section of the head at the base of the proboscis, (plate XVI, fig. 2) the latter appears to arise from a **U**-shaped mass under the clypeus, the upper parts of the arms of the **U** representing the genae or cheeks—narrow areas of the head situated in front of the eyes. The proboscis measures as a rule about three or four times the length of the head; in *Anopheles costalis*, 2 mm.

Parts constituting the proboscis

The proboscis consists of the upper lip—the labrum; the epipharynx; these two being firmly united together; the hypopharynx or tongue; two mandibles and two maxillae, which are commonly known as the stylets or setae, consisting almost entirely of transparent chitin, and used to pierce the skin; and the labium, or lower lip, the largest and fleshy part of the proboscis, in a groove on the upper surface of which the other parts are ensheathed when in repose. On either side and above the labium are the two maxillary palps, rod-like organs, covered with hairs and scales, and which, in *Anopheles*, lie above and parallel to the other mouth parts, and extend almost to the tip of the proboscis.

The general arrangement of the mouth parts to one another is seen in plate XV, fig. 3, a transverse section about the middle of the proboscis.

The epipharynx. The central tube through which the blood is sucked is formed by the epipharynx, which is morphologically the continuation of the upper and lateral chitinous walls of the pharynx. This tube is tunnel-shaped, being flattened on its under surface; its distal open end is oval, and looks ventrally—a fact first pointed out by Swammerdamm[1] in 1668. The wall of the epipharynx on the ventral surface becomes exceedingly thin, and fails to meet in the middle line, so that a slit is formed running the whole length of the epipharynx. The tip of the epipharynx ends

1. Swammerdamm, *Buch der Natur.* Leipzic, 1752.

in a sharp point, and presents the appearance of the point of a pen, having a central split and small eye. It is composed of two conical pieces, the bases of which blend with the upper rounded wall of the tube—and a slight thickening at the junction of the two pieces in the middle line gives rise to the appearance of the slit of the pen. On each side of the epipharynx, at its base, and intimately blend with it, is a stout rod of chitin having a core of large nucleated cells; this rod is the continuation of the lateral horizontal plate of chitin which at the base of the epipharynx affords attachment to the epipharyngeal muscles. The outer edge of it turns gradually upwards and inwards, and, fusing with the lateral convex surface of the epipharynx, forms the lateral supporting rod of chitin described. In transverse sections the core of nucleated cells in its interior is seen to be continued down the whole length of the epipharynx and at its distal end, the core turns upwards and towards the middle line; the epipharynx thus forming the extreme tip. The labrum, which is intimately blended with the epipharynx superiorly, thus takes no part in the formation of the extremity, stopping short before the nib-like tip is reached.

The interior of the epipharynx measures at its base, dorso-ventrally 19·8 μ, from side to side 26 μ; at the middle of the proboscis 16·5 μ dorso-ventrally, 18·1 μ across; and at the middle of the labellae 13·5 μ vertically by 13·2 μ across.

The **labrum** or upper lip is a delicate chitinous process situated immediately above the epipharynx and intimately connected with it, in fact it can be only partially separated from it by such reagents as caustic potash. For this reason DIMMOCK[1] described them as one piece—the labrum-epipharynx. The labrum arises at the base of the clypeus and runs along the upper surface of the epipharynx. In a transverse section near the base of the proboscis (plate XVI, fig. 1), it is seen that the labrum is composed of a curved lamella of chitin with its convexity approximated to the convexity of the upper surface of the epipharynx. The sides of the superimposed furrow thus formed, lower down the proboscis, suddenly become thinned, and, turning outwards and downwards are thrown into folds of very delicate chitin which unite below with the outer edges of the lateral rods of chitin of the epipharynx (plate XVI, fig. 1), the space thus closed in is occupied by loose cellular very delicate connective tissue. Towards its distal end, the furrow of the labrum becomes shallower and opens out, and the labrum itself becomes more intimately fused with the epipharynx. In sections (plate XV, fig. 1) near the tip of the proboscis the labrum-epipharynx is seen as a more or less triangular-shaped piece made up of three parts; two lateral pieces of chitin, in the centre of each of which is a deeply stained nucleus (chitin-cell); and a superimposed crescentic central upper piece united with the lateral portions by a very delicate band of tissue; this represents the tip of the labrum, which, as has been already described, stops short of the end of the epipharynx.

1. Dimmock, *The anatomy of the mouth parts and of the sucking apparatus of some Diptera.* Boston, 1881. P. 13.

At the proximal end the chitinous lamella of the labrum ends within the clypeus, projecting upwards for a considerable distance as a flattened rod-shaped piece, which affords attachment to fan-shaped muscles, arising from the roof of the clypeus.

The hypopharynx. SAVIGNY (1816); lingua, WESTWOOD; ligula, KIRBY and SPENCE (1828); or tongue, is formed by a prolongation of the chitinous lower wall of the pharynx. It is a thin, flattened lamella of chitin, closely applied to the under surface of the labrum-epipharynx. Its lateral edges are turned upwards slightly, and upon these rest the inner edges of the mandibles and the convex basal borders of the epipharynx. The tip of the hypopharynx is simple and lanceolate. In the centre of the hypopharynx the chitin is thickened and deeply hollowed out on its upper surface, to form an almost completely closed gutter running down the whole length of the organ, and approximated to the slit on the under surface of the epipharynx. The hypopharynx consists of an upper thick flattened plate of chitin, hollowed at its centre to form the gutter, and a lower thin plate; the intermediate space being filled with delicate connective tissue, and is lined with chitin forming cells: well seen in a section at the base of the proboscis (plate XVI, fig. 1). Throughout the distal two-thirds the two plates are fused together, the space remaining as a core of cells, imbedded in the chitin on each side of the salivary gutter. This gutter commences as a V-shaped opening (plate XIX, fig. 1) at the point of origin of the hypopharynx. Connected with this aperture is the salivary receptacle (plate XVIII, fig. 1 *s.r*), a hollow, cone-shaped organ, lying applied to the ventral wall of the pharynx. The base of the cone points backward and slightly upwards; the apex, after a slight curve upwards, opens on to the salivary gutter at the V-shaped slit. The sides of the receptacle are of thick opaque chitin, except on its dorsal surface, which is somewhat flattened and composed of thin membranous transparent chitin. The lateral walls of the receptacle are strengthened by chitinous bands from the lateral portions of the clypeus. The base of the receptacle is distinctly membranous in character, very faintly staining with haematein: a little below its centre the common duct of the salivary glands is inserted. Above and around the insertion of the duct are attached the fine tendons of two muscles, one from each side (plate XVIII, fig. 1 *f.m.* and XIX, fig. 1 *r.m.*). These muscles arise together from the ventral surface of the lower chitinous plate of the pharynx, but more especially from a chitinous ridge on each side, which is concave anteriorly and also from above down, and projects from its under surface near the junction of the first and second portions of the pharynx (plate XVIII, fig. 1), The mechanism of the receptacle is probably as follows :—When the muscles contract, dilatation of the cavity of the receptacle is produced by pulling of the membranous base outwards; saliva then flows in and fills the cavity. On relaxation of the muscles, the membrane springs back into its original position, thus expelling the saliva down the channel of the hypopharynx.

The mandibles, two in number, are extremely delicate, transparent scroll-like rods of chitin, applied, one on each side of the base and sides of the epipharynx,

they are concave on their inner and convex on their outer surfaces, and are of uniform thickness for the greater part of their length, but for a short distance above their sharply-pointed tips they broaden, become more lance shaped and are twisted once upon themselves. In a transverse section at this level, they present several concavities into which the sides of the labrum-epipharynx, hypopharynx and maxillae fit (plate XV, fig. 1). Near their termination on the outer convex surfaces, lying along the upper edge is a row of very fine sharply pointed teeth varying in number, the sharp points projecting downwards. DIMMOCK[1] does not describe these teeth-like processes as occurring in the three species of *Culex* on which his observations were made. With regard to the origin of the mandibles DIMMOCK[2] says 'at the base of the proboscis they appear to have no muscular attachment but to lie embedded in the connective tissue beneath the pharynx and above the maxillae.'

In *Anopheles costalis*, plate XVI, fig. 2 shews their close relation to the inner surface of the base of the maxillary palpi, as a straight piece of chitin enclosing delicate cellular tissue. In sections further back they are difficult to trace but appear to come into relation with a downward projecting plate of chitin about the level of the anterior edge of the gena. They would thus appear to arise from chitin in the close neighbourhood of the groove between the clypeus and the gena. By tearing away the parts of the proboscis by traction at the tip with the finger nail, the mandibles come away with the maxillae attached to the maxillary palpi. To the base of each mandible a muscle is attached by a fine tendon ; the muscle arises from the ventral surface of that part of the chitinous exoskeleton of the head, which is folded inwards beneath the eyes ; the fibres are directed forward and slightly downwards.

The maxillae are two stouter lancet-shaped processes of chitin, one on each side ; concave on their inner surface and fitting beneath the sides of the mandibles and the hypopharynx. On the upper and inner surface a slight distance from its inner edge runs a stout ridge of chitin from which the thinner portion of the maxilla curves upwards and outwards. The stout ridge is continued to the distal end of the maxilla, forming the sharp point. Some little distance from the point of the maxilla the thinner portion begins suddenly to shade off like the sharp edge of a penknife ; this surface bears on its ventral side near the outer edge fifteen to twenty low conical chitinous papillae. DIMMOCK[3] refers to them as being on the dorsal surface in *Culex*, and says 'they are true papillae, not points of a serrate edge.' The thinner portion of the shaft of the maxilla is marked with alternate light and dark bands at right angles to its longitudinal chitinous rod ; this is due to the fine corrugation of its surface pointed out by DIMMOCK.[4] This appearance is not

1. Dimmock, *The Anatomy of the Mouth parts, etc., of some Diptera.* Boston, 1881.
2. Dimmock, *Loco-cit*, p. 16.
3. Dimmock, *Loco cit.*, p. 17.
4. Dimmock, *Loco cit.*, p. 16.

present on that portion of the maxilla from which the papillae arise. In a transverse section of the maxillae near their tips they present a peculiar jaw-like shape (plate XV, fig. 1). The maxillae appear to arise from the under surface of the maxillary palpi between them and the upper outer surface of the labium; in transverse sections they appear in this region as two lateral sickle-shaped stout masses of chitin, situated on each side of the commencement of the hypopharynx (plate XVI, fig. 2). Each maxillae is continuous with a thick rod of chitin, which extends almost the whole length of the head, ending in a long upper and a lower shorter stumpy rounded process in the basal part of the occipital region. To these intercranial chitinous rods, which appear to lie free in the cellular tissue at the base of the head, powerful muscles connected with the movements of the proboscis are attached (plate XIX, fig. 1; XVII, fig. 1; and XVIII, fig. 1). These processes have been variously termed; by Lowne,[1] 'apodémes;' by Macloskie,[2] the 'great tendons' of the mandibles. Gerstfeldt[3] regarded them as the basal portions or 'cardines' of the hypopharynx. No mention is made of them by Packard[4] in his description of the insects' mouth parts. Smith[5] regards these basal processes found in various genera of diptera *Bombylius*, *Anthrax*, *Eristalis*, *Musca*, etc., as basal prolongations of the palpifers (the mandibles of other authors), and states they may perhaps represent the 'stipides' as well—which he has not as yet identified in the dipterous mouth parts. It would seem from Dimmock's[6] account of the anatomy of *Culex* that these chitinous rods do not extend so far back into the head in this genus; he says 'their continuations (of the maxilla) in the head are two delicate chitinous supports, each of which ends in a strong muscle; this muscle—the retractor maxillae—passes backwards and downwards through the head beneath the infra-oesophageal ganglion, and has its origin in the posterior basal part of the head.' That they are the supports from which the maxillae arise can be well seen in serial sections.

The muscles in connection with the chitinous intra-cranial processes of the maxillae are:—

1. Muscles fixing them to the cranial exoskeleton. Each has a large muscle which arises from the lower occipital region of the cranium and is attached to the outer side of the process for the greater part of its extent: the fibres of this muscle run horizontally; muscle fibres, directed upwards, also run in connection with the terminal bifid extremity and that portion of the exoskeleton of the head, which is folded beneath the eyes (plate XIX, fig. 1 *z.m.*).
2. A spindle-shaped belly of muscle arises from the ventral surfaces of the processes to be inserted into the base of the labium (plate XVII, fig. 1; XIX, fig. 1 *l.m'*).

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1. Lowne, *The Anatomy and Physiology of the Blow-fly*. London, 1870.
2. Macloskie, *The Proboscis of the House-fly*. American Naturalist, 1880, vol. xiv, p. 153.
3. Gerstfeldt, *Ueber die Mundtheile der saugenden Insecten*. Dorpat, 1853.
4. Packard, *Text-book of Entomology*. New York, 1898.
5. Smith, *Trans. American Entom. Soc.*, vol. 17, 1890, p. 338.
6. Dimmock, *The Anatomy of the Mouth parts, etc.* Boston, 1881. P. 16.

3. Muscle fibres arise from the superior surfaces of these processes, from a short length at their distal ends, and are directed upwards and forwards to be inserted into the upper surface of the first joint of the maxillary palpi.

Though the stylets appear to be wholly of chitinous structure yet in transverse section at their point of origin (plate XVI, fig. 1) it is seen that they really consist of a central prolongation of the delicate tissue lining the head, encased in a thick chitinous envelope under which is a row of flattened cells with large deeply staining nuclei ; these cells which secrete chitin can be traced almost to the tips of the labrum-epipharynx and hypopharynx. In the maxillae and mandibles traces of these chitin cells are seen in sections near the tips as a central staining core. (Plate XV, fig. 1 and 2). It is to be remarked how the shape of the stylets serve to bind them together, the convexity of the one above fitting into the concavity of the one below ; thus forming a solid chitinous awl with which the skin is pierced. A section at the tip of the proboscis illustrates the fitting of the stylets with one another (plate XV, fig. 1). In fact if the tip of the proboscis be cut off a little above the labellae, the stylets fall out of their labial sheath as one piece, nor do they separate unless pressure be applied. A good view is obtained in this way of the saw-like edges of the mandibles and maxillae, the latter being below and to the outside of the former.

The maxillary palpi. In *Anopheles*, the maxillary palpi are two long segmented rounded processes, thickly covered with hair and scales, lying in the resting condition,one on either side, on the upper surface of the labium and its enclosed stylets; their tips are rounded off and end a little short of the tip of the proboscis. They are attached to the side of the head below on either side of the clypeus, their under surfaces here being in close relation to the maxillae (plate XVI, fig. 2). The basal joint is bulged on its upper surface ; on its under surface the chitin is thickened to form a ridge, which is in close relation at its proximal end to the chitinous prolongation of the maxillae. This joint contains muscle fibres arising from the maxillary prolongation ('great tendon') near its union with the maxilla, which are directed obliquely upwards and forwards to be inserted into the bulged upper surface.

Each palpi contain delicate connective tissue containing large cells: a nerve, comparatively large, arising from the lateral surface of the infra-oesophageal ganglion, and numerous very small tracheae. Muscle fibres are only present in the basal joint. The study of the maxillary palpi with regard to shape, size and surface markings is of great importance in the classification of species.

The labium or lower lip is the largest of the mouth parts and acts as a sheath for the stylets. It commences as a free piece in the same plane as the other mouth parts and is a continuation of the lower anterior part of the head below the pharynx. On its under convex surface it is marked off from the ventral surface of the head by

a slight groove which continues upwards on either side for a short distance, becoming deeper. Its convex ventral and lateral chitinous surfaces are thickly covered with hairs and scales, the chitin bearing them having irregular annular markings. Its upper surface is of smooth chitin, upon which the stylets rest. The labium tapers slightly from base to apex: at its commencement it is broad from side to side, its internal measurement from above down being 45·6 μ; from side to side 65·2 μ; its smallest depth is 22·8 μ and width 42·4 μ. Its upper smooth surface is here flattened and on it rest centrally the hypopharynx, on either side the two maxillae (plate XVI, fig. 2). A little way from its origin, the labium becomes roughly round in shape owing to the edges of the upper surface turning upwards and inwards over the stylets forming a large oval channel in which they lie (plate XV, fig. 3); these edges are extremely fine and do not meet in the middle line, so that a space of uniform width is left running along the dorsal surface of the labium to its extreme tip. At about 0·16 mm. from the extremity of the proboscis the labium proper ends abruptly, while its upper concave surface is continued on to the tip of the proboscis, gradually tapering to a blunt point covered with fine hairs. This tip of the labium is easily broken off in dissections of the proboscis.

At the abrupt ending of the main portion of the body of the labium, which in transverse section is somewhat oval from side to side, are attached by true joints two lobiform appendages—the labellae—which enclose between their inner surfaces the tips of the stylets and the true tip of the labium. Crescentic at their bases, the labellae gradually taper to form the tip of the proboscis.

Running longitudinally on each side of the labium, and projecting into its substance from the inner surface of the chitinous exoskeleton, is a thick, very opaque chitinous ridge. These ridges commence at the base of the labium, and end abruptly a little distance behind the point of attachment of the labellae (plate XIX, fig. 3); from their inferior surfaces for about 34·3 μ from their distal extremities, and extending obliquely downwards and upwards, the chitin of the convex under surface of the labium proper becomes greatly thickened, forming two ventral plates, which in the mid-ventral line curve upwards and outwards, scrollwise, into the substance of the labium, ending in a short thick rod, near the centre of each lateral half of the labium (plate XIX, fig. 3 *r*). They present four borders: a proximal convex border continuous with the general exoskeleton of the labium; an outer border limited by the lateral longitudinal ridges of the labium; an inner, ending abruptly in its substance as a thick ridge of chitin; and a lower distal border, convex, curving from within, outwards, and upwards towards the distal end of the lateral ridge of the labium: upon the thickened inner extremity of this surface, which is hollowed out for its reception, the labella articulates. The labellae being removed, a view of the termination of the labium seen in section (plate XV, fig. 2) presents the following regions: on either side a pear or kidney-shaped area, approximated below in the median line to its fellow; to these areas the bases of

the labellae are applied. Above and resting between these areas is the concave tip of the labium (seen as a concave band of chitin in section), on which the stylets rest: these three parts enclose a roughly triangular area covered by a delicate membrane, thrown into folds, and extending above along the under surface of the tip of the labium, fusing with its sides and tip; on either side being in connection with the bases of the labellae and with the joint. This membrane bears a few very fine hairs, and it probably allows of considerable play when the labellae are separated; with them it touches the skin when the mosquito sucks blood, being then stretched to some extent.

The labellae are conical and roughly crescent-shaped in section; their apices form the extreme tip of the proboscis. They present two surfaces, an outer convex, an inner concave; and two borders an upper and a lower, the former being in the same line as the edges of the upper surface of the labium.

In some species of *Culex* and in *Anopheles maculipennis* they consist of two parts, a distal, and a basal upon which the distal half is jointed to allow of some outward movement; the joint being represented by a narrow white line beginning near the apex on the outer surface at its upper border and curving sharply downwards and outwards to the lower border; about the centre of this line is a sharp upward bend. This peculiar division of the labella is absent in *Anopheles costalis*. SMITH[1] who points out the homology of the so-called labium of the *Diptera* with the galea of other insects, states with regard to its tip in five species of *Culex* he examined 'no two agreed in structure.' We have found this to be the case also in a few species of mosquitoes we have examined, especially with regard to the structure of the joint at the base of the labella.

The outer surfaces of the labellae are covered with fine hairs and here and there coarser ones. The inner concave surfaces are marked by longitudinally ridges and folds. There are no 'pseudo-tracheae.' In transverse section (plate XV, fig. 1), three regions are distinguished; an upper somewhat flattened, the chitin of which is very thin and thrown into numerous small folds from which arise a felt-work of exceedingly fine long hairs, crossing one another in all directions; a lower area of fairly thick chitin limited by the rounded inferior border of the labella: from it arises long thick bristle-like hairs projecting downwards in between the tips of the labellae. These two areas, well marked near the tips of the labellae, gradually fade away towards their bases. Between them, the central region is deeply hollowed out and ridged and folded, its chitin is much thicker and free from hairs. A little below its centre, running longitudinally down this surface is a stout ridge of chitin which can be traced in a cleared specimen of the labium, mounted whole, to the base of the labella (plate XIX, fig. 3): here it makes an outward curve to about the centre of the

1. Smith, *Trans. American Entomol. Soc.*, vol. xvii, 1890, p. 330.

base, and turning sharply back, turns upwards for a short distance to terminate in a rounded knob, which articulates with the chitinous surface described at the distal end of the labium. To the outer bend of this rod the tendon of the muscle of the labella is attached. When these muscles contract, the labellae are drawn apart and rotated in such a way that their inner surfaces look downwards: it is probable that only the anterior distal portion of their inner sufaces is applied to the skin.

The internal structure of the labium. In a section of the proboscis about its middle (plate XV, fig. 1) it is seen that the chitinous exoskeleton of the labium is lined with a delicate spongy tissue containing very large rosette-shaped cells—a continuation of a similar tissue lining the cranium. Beneath the chitinous envelope, here and there, is a row of low cubical epithelial cells (hypodermis). Situated about the centre of the section are the two labial tracheae, one on each side side, each surrounded by a delicate cellular sheath; with each runs a comparatively large nerve-trunk—the nerves to the proboscis. The tracheae are the terminal branches of the large tracheae to the head; they join the nerves to the proboscis immediately after their origin from the suboesophageal ganglion: on their way down the labium they give off small lateral branches and becoming smaller, eventually break up into innumerable fine branches about the lower third of the labium to supply the labellae. The nerves, two in number, are the main anterior branches of the suboesophageal ganglion. Running on either side of the common salivary duct they enter the labium beneath the salivary receptacle on the under and outer side of the tracheae, being closely applied to them: after a straight course they split up in the labellae into many fine fibres which are distributed over their inner surfaces.

Internal structure of the labellae. Applied to the outer wall and bulging into the interior of each labella, almost completely filling it is a mass of deeply staining tissue which, with a high power, is seen to be composed of numerous cells very similar in shape and size to the nerve cells of the supra- and infra-oesophageal ganglia of the head. Over the surface of this densely cellular mass, the nerve to the proboscis ends by splitting up into fine filaments (plate XIX, fig. 2). The close relation of the nerve to the proboscis to this structure, points to its being ganglionic in nature, probably supplying the numerous sensory hairs on the inner surface of the labellae with nerve fibres. These ganglia are well supplied with very fine tracheae, the terminal branches of the tracheae to the proboscis.

Muscles of the labium are of two sets:—

Those attached to the base of the labium.

Those arising within the labium.

The latter—the muscles of the labellae—are two long slender paired muscles, each arising by numerous separate bundles of fibres from the dorsal and ventral surfaces of the lateral chitinous ridges of the labium; they are directed very

obliquely towards the tip of the proboscis. These bellies of muscle end in minute tendons which join a very long common tendon, running parallel to the chitinous ridges and extending the whole length of origin of the muscle. These long tendons do not quite reach the mid-line of the labium (plate XIX, fig. 2, *l.m'*); becoming somewhat thicker, they are eventually inserted into the bases of the labellae, chiefly at the chitinous angle mentioned above. These muscles do not appear to take origin from the basal third of the chitinous ridges of the labium. DIMMOCK[1] describes in *Culex* two muscles in relation to each labella, a flexor and an extensor, the flexor being to the inner, the extensor to the other side of the cavities of each lobe, and having origin within the head.

Muscles attached to the base of the labium. One pair of muscles is attached directly to the base of the labium. These are a pair of spindle-shaped muscles, each of which arises from the under surface of the basal chitinous support of the maxilla and is inserted into a ridge of chitin projecting from the groove which separates the labium from the under surface of the head (plate XIX, fig. 1, and XVII, fig. 1, *l.m'*). DIMMOCK describes these muscles in *Culex* as extending along the labium.

The clypeus, or epistom, is the anterior projecting hood-shaped portion of the face from which the proboscis is suspended. It is limited above from the rest of the head by a deep groove; behind and to the right and left of this groove arise the antennae which are to some extent supported by the upper surface, this being slightly hollowed out for the reception of their basal joints; at the sides and posterior are the genae or cheeks, separated from the clypeus by grooves. In transverse section the clypeus appears as a blunt, wedge-shaped piece, the thinner end of which is formed by the upper wall of the pharynx (plate XVI, fig. 2), surrounded below and its sides by a U-shaped area (plate XVII), which for the most part eventually breaks up into the parts forming the proboscis.

From the anterior wall, and from that part of the under surface of the clypeus which forms the roof to the labrum at its origin, project two plates of chitin (endosternites) for some little distance (plate XVIII, fig. 1, *f*): these are approximated below and have an upper and a posterior free edge and two surfaces, inner and outer; the upper posterior angle is lengthened into a blunt process (plate XVI, fig. 2, *f*). These plates are homologous to the fulcrum of other Diptera—for example, *Musca*, *Eristalis*—which have a proboscis capable of extension and retraction. The fulcrum of such Diptera is greatly developed, and moves around an axis at the anterior angle of the head, and encloses the pharyngeal muscles. In the Culicidae the proboscis is fixed in a more or less permanently extended position, and the fulcrum is ill-developed and firmly attached to the anterior wall of the head.

The inner walls of the clypeus afford attachment to three sets of muscles :—

1. Muscles in connection with the labrum.
2. Muscles to the base of the epipharynx.
3. Muscles in connection with the pharynx.

1. Dimmock, *The Anatomy of the Mouth parts, etc., of some Diptera.* Boston, 1881. P. 18.

The muscle attached to the labrum on each side consists of two bundles of fibres lying side by side, having an extensive origin from almost the whole of the upper median surface of the clypeus from before backwards. Their fibres are directed backwards and collect together in a fan-like manner, to be inserted into the projecting chitinous base of the labrum (plate XVIII, fig. 1 and XVII, fig. 1 *lbr.m.*).

The muscles in connection with the base of the epipharynx are two lateral groups arising from the lateral outer wall and free edges of the fulcrum ; a few fibres probably arising from the adjacent inner wall of the clypeus. The fibres project vertically downwards, and are inserted into the horizontal plate of chitin on either side of the epipharynx (plate XVI, fig. 2 *e.m.*)

The third set of muscles arise from the upper inner surface of the clypeus on each side of the labral muscle mass ; the fibres run backwards and downwards to be inserted into the upper chitinous plate of the ascending portion of the pharynx—each muscle being divided into a central and two lateral portions, inserted into the central membranous and anterior and posterior chitinous portions of the wall respectively (plate XVIII, fig. 1, and XVII, fig. 1 *p.m.*). The remainder of the clypens is occupied by tracheae and nerves for supply of the above muscles, and it is lined by loose spongy fatty connective tissue.

The pharynx is that part of the alimentary tract, lined with chitin, which extends from the base of the proboscis to the commencement of the oesophagus at the junction of the head and neck. It consists of two portions, a short anterior ascending and a longer horizontal portion, the latter passing through the ganglionic ring formed by the supra- and infra-oesophageal ganglia and their commissures. Here it forms a large chamber—the pumping organ. DIMMOCK[1] describes this part of the pharynx as the oesophagus. The first part of the pharynx is narrow and is a tubular continuation of the epipharynx above and the hypopharynx below ; it passes upwards and backwards, ending opposite the furrow separating the clypeus from the head. Here the pharynx suddenly turns backwards and is continued on as the second part of the pharynx. The first part of the pharynx consists of two plates of chitin, an upper and a lower ; the former limits the clypeus internally; it is not completely chitinous, in fact only its anterior and posterior portions are chitinised and thin off towards the centre of the plate which consists of a membrane covered with flattened epithelial cells (plate XVIII, fig. 1, and XVII) ; to this membrane are attached the oblique central fibres of the pharyngeal muscle. On the pharyngeal surface of the anterior chitinous portion of this upper wall of the pharynx are a few low conical papillae (taste papillae) (plate XVIII, fig. 1). The posterior upper edge of this wall is curved slightly outwards upon itself and is attached to the upper wall of the second part of the pharynx by a folded band of chitin.

The ventral wall of this part of the pharynx is a stout plate of chitin, anteriorly continuous with the hypopharynx, posteriorly with the ventral wall of the second

1. Dimmock, *The Anatomy of the Mouth parts, etc.* Boston, 1881. P. 13.

portion. Anteriorly and laterally it is curved upwards, and unites with the sides of the clypeus (plate XVII, fig. 1). From its under surface near its posterior edge it gives off on each side a hook-like ridge of chitin (plate XVIII, fig. 1, *x*) from which the muscles of the salivary receptacle have origin. Dimmock describing the pharynx states 'the channel for the passage of food turns upwards and then backwards again, passing in its course a place where its wall approximate dorsally and ventrally; this narrowing of the walls is probably a valve to prevent the return of fluids to the mouth during the pumping process.'

In *Anopheles costalis*, situated in this position and attached to the upper surface of the slightly horizontally bent posterior end of the ventral chitinous plate, is a peculiar ridge of chitinous stout hair-like processes, which curve forwards so that their tips lie in the angle between the upper surface of the first and second parts of the pharynx. The hairs are of two kinds, an anterior large set—probably a single row—and a posterior, small, fine set situated in a clump immediately behind the former. The larger hairs consist of a short stout shaft firmly embedded in the chitinous pharyngeal wall; this shaft supports a cup with a free rim curved outwards; within the cup lies the oval-shaped bulbous extremity of the base of the hair; this bulbous extremity contains a single large cell. The remaining free portion of the hair curves forwards and tapers to a fine point, and appears to have a central shaft enclosed within a chitinous cuticle from which barb-like processes project. The hairs of the posterior set are much finer and shorter, and are more numerous; they appear to be simple in character. In transverse section (plate XVIII, fig. 2) this structure presents to some extent the appearance of 'rods and cones.' The suboesophageal ganglion lies in close proximity to this structure, but no nerve fibres have been traced to communicate with these specialised hairs, although such probably exist. That in the first place these hairs act in conjunction with the general conformity of this part of the pharynx as a valve to prevent the regurgitation of blood back into the mouth during the action of the pumping organ seems to admit of no doubt; on the other hand such specialisation in structure would lead one to suppose that they possess also a sensory function.

The mechanism of the proboscis. The mosquito, when alighting on the surface of the skin for the purpose of sucking blood, immediately raises the palpi almost at right angles to the proboscis. After probing about with the labellae for a suitable spot to pierce the skin, it plants them firmly on the surface, the proboscis being directed a little forwards. A moment later the labium is seen to bend backwards near its junction with the head, the stylets, remaining straight, becoming thus uncovered. The bending of the labium becomes more marked as the stylets sink into the skin, the angle of the bend travelling towards the middle of the length of the proboscis, so that when the stylets have entered the skin to nearly their full extent, the labium is bent double beneath the head of the insect. Reaumur was the first, probably,

to describe and figure the manner in which the labium was disposed of during the puncture of the skin. The stylets probably enter the skin as one piece, being guided by the tip of the labium and supported on each side by the basal portions of the labellae. The piercing of the skin is brought about by muscular force directed from the body of the insect, the muscles attached to the bases of the stylets serving to keep them rigid. The withdrawal of the stylets is accomplished by the powerful retractor muscles attached to the chitinous prolongations of the maxillae, and the muscles described in connection with the bases of the other mouth parts. During the process of extraction, while the stylets are slowly sinking into the groove on the upper surface of the straightening labium, the insect keeps the labellae pressed firmly upon the skin. After they have emerged, the labellae spring together over their tips.

By a careful study of the minute anatomy of the proboscis, as detailed above, it is not difficult to suggest a method by which the mature larvae of *F. nocturna* may escape from the proboscis. As above mentioned the dimensions of this larva are 1·006 mm. long and 0·025 mm. broad. It is therefore evident, taking into consideration the dimensions of the several parts of the proboscis, that the most likely method of gaining access to the proboscis from the head is by entering the body of the labium, the structure and disposition of which would easily admit of this. It has been suggested that the larvae lie among the stylets—in which case it will be seen from the study of the attachments of these appendages that the larva would in its course, necessarily have to pierce a stout layer of chitin, a procedure exceedingly improbable. But the evidence that the larvae do reach the labium is now conclusive. Low[1] in sections of the proboscis found them there ; and although he describes them as 'making an independent passage through the base of the labium and pushing forward along the proboscis between the labium and the hypopharynx amongst the stylets, where they are found stretched along the length of the proboscis head foremost,' the illustrations of his sections of the proboscis shew the worm in the body of the labium, and he cannot have been intimately acquainted with the minute and most delicate anatomy of these parts. These illustrations certainly do not shew the worm 'amongst the stylets,' but in the tissue of the labium.

Grassi and Noe[2] often found *F. immitis* in the labia of mosquitoes (*Anopheles claviger*) which had fed on the blood of an infected dog ; and we ourselves, once in a dead mosquito, and again in a living insect, found the larvae alongside the tracheae of the labium.

The question then arises as to how the larva leave the body of the labium and reach man, since it must be presumed that their presence in such an organ as the proboscis indicates that they subsequently leave that organ during or about the time of puncture. Judging from the condition of the larva at this stage, which

1. Low, *British Medical Journal*, 1900. Vol. II, June 16.
2. Grassi and Noe, *British Medical Journal*, 1900. Vol. II, p. 1306.

shows a complete alimentary canal and reproductive apparatus (although immature) similar in site and arrangement to those of the adult worm as found in man, it seems certain the next stage in the life history is carried out in the definitive host—man. It has been suggested, that as mosquitoes can be sometimes observed feeding on such as bananas, that the filariae are capable of exercising a selective instinct for their escape at the time of puncture: and it has been further suggested that possibly the filariae may escape into banana and other food stuffs, and either undergo a further period of their life history in the external world, or without further change be introduced into the alimentary tract of man. All these suggestions appear to us exceedingly improbable. We have previously shewn,[1] and there is a considerable amount of other evidence to support the facts, that a fertilized female mosquito of the blood-sucking species of West Africa requires blood regularly for the maturation of her ova, and that she will have blood and nothing else : and since those species capable of carrying human filaria frequent the neighbourhood of human habitations, they will for the whole period of their existence feed on blood, and generally on human blood—so that the possibilities of the escape of the filariae into banana and other substances are extremely vague, and further, it becomes quite unnecessary to suppose the possession by the larvae of any selective instinct. The occurrence of the larvae in such a position leads one to presume that they leave it before, during or after the act of suction of the blood; and GRASSI and NOE[2] claim to have infected a dog by the bites of *Anopheles* infected with *F. immitis*, although, since a single broken immature worm only was discovered, *post-mortem*, some sixteen days after the mosquitoes had been allowed to bite them, this experiment urgently requires confirmation. These investigators, however, assert that in specimens of the numbers of mosquitoes which were allowed to bite the dog, before the experiment, larvae were found in their labia, while after the experiment, many labia were dissected and found empty.

GRASSI and NOE in their article go on further and describe how the larvae leave the labium. After drawing attention to the bending of the labium, as the stylets gradually penetrate the skin, so that the angle formed advances from near the base to the middle of the labium until the labium appears almost completely doubled, they 'add the two halves of the olive and the little tongue resting against the skin of the animal, which is punctured, embrace the six pieces penetrating the skin. It is certainly through the bending of the labium, stuffed with filariae, that is brought about the rupture of the integuments of the labium along the dorsal groove, and through the rupture thus produced come out the filariae to penetrate the body of their definitive host. It is difficult, as everyone will understand, to enter into further particulars. In some cases we believe that we positively found the rupture in the middle of the length of the labium in correspondence with the loop. It seems to

1. *Report of Malaria Expedition to Nigeria*, 1901. Part I, chap. iv.
2. Grassi and Noe, *British Medical Journal*, 1900. Vol. ii, p. 1306.

us also that the two halves of the olive and the little tongue being in the above-mentioned position have an importance in directing the movements of the filariae towards the wound made by the stylets. Perhaps the gases emitted in the first moment of the bite help the entry of the filariae into the body of the definitive host.'

One cannot read this paragraph without being struck with the remarkable ingenuity displayed in its account of how the filariae leave the proboscis of the mosquito. But a very careful and exhaustive study of the structure and relations of the parts forming the proboscis has convinced us of the utmost difficulty the most inquiring of observers would experience in deciding the occurrence of any such slit in the upper surface of the labium, as the authors believe they have seen. Furthermore, the upper surface of the labium is composed of chitin almost as thick as that on the outer surface (plate XV, fig. 3). Moreover, from the illustration of the longitudinal section of the proboscis accompanying Low's article, it appears to us that the head of the filaria in the labium is considerably beyond the middle of the labium, in fact appears to reach the distal end of the labium proper—as MANSON[1] says, 'to the tip of the proboscis.' Such a position, if the filariae escape in the manner GRASSI and NOE imply, would necessitate their exit, middle part first, at the bottom of the very acute angle formed by the two almost completely folded parts of the labium. The difficulties involved in such a method of exit appear to us insurmountable.

Referring again to the structure of the extreme tip of the labium (page 80), we have stated that at about 0·16 mm. from the tip of the proboscis the labium proper appears to end bluntly but its upper surface is found to continue on, gradually tapering to a blunt point covered with fine hairs. And again (page 81), above and resting between these areas (lateral areas on the end of the labium proper) is the concave tip of the labium (seen as a concave band of chitin in section) on which the stylets rest ; these three parts (the two areas and the concave chitinous band) enclose a roughly triangular area covered by a delicate membrane thrown into folds ; above it extends along the under surface of the tip of the labium, and on each side is in connection with the bases of the labellae.

When the tip of the proboscis is applied to the surface of the skin, it has been seen that the two labellae swing apart and are rotated so that their inner surfaces are in contact with the skin, and that the piercing stylets are directed in their course by the concave upper surface of the extreme end of the labium. By the swinging of the labellae the delicate folded membrane is somewhat stretched and is close to the surface of the skin. This membrane is exceedingly delicate so that in transverse section even with the high powers of the microscope ($\frac{1}{12}$ O.E.) the sections of its folded edges are represented by thin fine lines. It will thus be seen that this is the most delicate part of the labium ; and as both Low indicates in his illustration and

1. Manson, *Tropical Diseases*. London, 1901. P. 496.

MANSON[1] asserts to have often observed, the head of the mature larva (in fact there appear to be more often a pair) is in the immediate neighbourhood of this spot; it is extremely probable that the larvae escape by the rupture of this thin membrane, which is probably already stretched by their presence, when the labellae swing out, and stretch the membrane still more. The escape of the larvae in this way may possibly be aided as the bend of the labium travels from the base towards the middle of that organ.

The relation between F. nocturna and F. diurna

The many points of resemblance between the embryos of these two worms suggest the question of their identity, and in favour of the view of their identity many facts can be brought forward. In consequence of the importance of the subject, and the many points of interest involved therein, we propose to treat of the arguments for and against in some detail; and to arrange them under some chief headings.

Geographical distribution. As has been already pointed out, the distribution of elephantiasis (caused by the presence of the adult form of *F. nocturna* in the lymphatic vessels and other sites) is extremely wide; but limiting ourselves to the distribution of *F. nocturna*, as determined by the presence of embryos in the blood, it corresponds in certain regions with that of *F. diurna*—the two occurring side by side throughout large tracts of country. On the other hand, however, there appear to be many lands where *F. nocturna* alone is found; but as far as is at present known, in no district has it been shewn that *F. diurna* prevails alone. Reference must again be made in this connection with the conditions occurring in some of the islands of the Pacific, already mentioned, where elephantiasis is very prevalent, and an embryo occurs in the blood of many natives, which resembles very closely *F. nocturna*, yet shews none of its characteristic periodicity.

The microscopical appearances of the embryos. It has already been stated that in West Africa we were unable to distinguish the embryos in the blood of natives infected with *F. nocturna* and *F. diurna* respectively, by any means whatever. They appeared identical in their appearance, characters, measurements and movements in fresh preparations and correspond in length, breadth, staining reactions, and in the possession of the same number of 'spots,' situated at similar points along the length of the worm and of the same shape and size. The sheath, a common feature of each, appeared identical. Moreover, the West African *F. nocturna* resembles very closely that of China and India as described by MANSON.

The numbers in peripheral blood. Here again there is a close similarity between the two worms. An ordinary case of either infection presents from twenty to sixty embryos in a drop of blood from the finger, at the time when the maximum number is present in peripheral blood—although in each case so many as four to five hundred may be present in exceptional infections.

1. Manson, *Tropical Diseases.* London, 1901. P. 496

Periodicity. It was this phenomenon, and this alone, which led MANSON to regard *F. nocturna* and *F. diurna* as distinct species. And certainly, in the limited condition of the knowledge of the subject, it was a very natural conclusion, one large set of cases which had been examined, shewing a characteristic periodicity with a maximum number of embryos present in peripheral blood at midnight, and a smaller set presenting the reverse conditions, a maximum number at midday. The departure from this interesting regularity to be first noted, was recorded by THORPE in the Tonga Islands where a large percentage of the adults shewed symptoms of elephantiasis, and where an examination of a large number of natives proved the presence of embryos in their peripheral blood both during the day and during the night in approximately equal numbers, and moreover shewed that the embryos were present throughout the whole of the day.

We have already given details of several cases illustrative of the same conditions (table VII), and furthermore we have shewn (tables VIII and X) that cases of filarial infection occur in whom the hour at which the maximum number of embryos is present in peripheral blood is not mid-day and midnight, but may be any other hour—3, 6, or 9 a.m. or p.m. And besides we have shewn that 'pure' cases of *F. diurna* and *F. nocturna* are considerably less frequent in West Africa than these irregular cases.

The definitive hosts. THORPE, probably bearing in his mind the classical experiment of MACKENZIE, and the repetition of that experiment in another case by MANSON, by which it was proved that by a change in the habits of a case of *F. nocturna*, the periodicity of the embryos could be completely inverted, becoming thus similar to that of *F. diurna*, explained the peculiar phenomenon of the occurrence of the embryos in the blood of the natives of the Friendly Islands by the habits of the natives, which he thus describes from MARINER's classical account of the Tonga Islands :

'The natives employ themselves in conversation not only at any time during the day but also at night. If one wakens, and is not disposed to sleep again, he wakens his neighbour to have some talk. By and by, perhaps they are all aroused, and join in the conversation. It sometimes happens that the chief has ordered his cooks in the evening to bake a pig or some fish and bring it hot in the middle of the night with some yams. In this case the torches are lighted, and they all get up to eat their share, after which they retire to their mats ; the torches are put out, some go to sleep, and others talk perhaps till daylight.'

Similar habits are in practice among the natives of the whole of West Africa, but to a larger extent and on a larger scale. We were often told by natives from different parts of the Coast that it is common practice in the respective countries to which they belong, to sing and dance the whole night through, especially on moonlight nights. In fact we have ourselves heard the midnight orgies in the native

towns which we visited, and especially of the Kroo boy gangs in the towns of Southern Nigeria. Moreover, we often observed, especially in those towns where civilisation was very backward, the natives asleep during the middle hot part of the day; indeed, the Kroo boy in English Government employ steals a mid-day nap whenever he can. These habits have been practised, no doubt, for generations, and probably were prevalent to a much greater extent for years before the influence of Europeans was felt. Such conditions would, in a great measure, account for the variety in the cases of filarial infection we met with in West Africa, and which THORPE observed in the Friendly Islands, and point strongly to the identity of the two embryos, or rather to the phenomenon of the accommodation of the one or the other or of an original embryo perhaps exhibiting no periodicity whatever, to the varying habits of the natives who formed their habitat.

The intermediary host. *F. nocturna* has been successfully cultivated in several species of mosquitoes of both genera. In West Africa, after several attempts, we were able to cultivate this embryo in *Anopheles costalis;* but all our efforts to cultivate *F. diurna* failed. But this is not remarkable, for, if *F. diurna* had been evolved in consequence of the habits of the natives, it is not unnatural to expect that its intermediary host is an insect, probably a mosquito, not essentially nocturnal in its habits such as *A. costalis*, but one whose habits are diurnal.

Analogy with avian filariasis. In the chapter on Avian filariasis we describe eleven new species of filariae, each having a different embryo; in fact, we were soon able after a little practice to decide the species of the worm even by a study of the stained specimen of the embryo. Each species then possesses distinct adults, which give rise to a characteristic embryo. This would suggest a similar condition among human filariae, and thus that *F. diurna* and *F. nocturna*, being indistinguishable in fresh and stained specimens, have a common adult form.

The adult form. The adult of *F. nocturna* is well known—*F. bancrofti.* The adult of *F. diurna* has not yet been described, unless *F. loa* be that form. Now, the distribution of *F. loa* is, as far as we can ascertain, limited to the West Coast of Africa, and MANSON makes the same statement. It has not been met with in any other part of the world,* and the occurrence of a worm of the length of *F. loa* occurring under the conjunctiva of the eye, cannot possibly have been overlooked anywhere.

F. diurna, as far as we at present know, is also apparently limited to the West Coast of Africa, and has been found in some cases of natives in which *F. loa* has been removed from the eye—although this is not remarkable as anything more than an ordinary coincidence, considering the prevalence of *F. diurna* cases on the Coast. Moreover cases of *F. loa* have occurred in which no embryos could be demonstrated in the blood.

* Stossich states that it occurs in the Antilles and Guiana, but Manson says, in his latest edition of *Tropical Diseases*, 1900, 'it is peculiar to the West Coast of Africa.'

The conditions in the Friendly Islands, previously often referred to, may perhaps be quoted as an exception to the statement above—that *F. diurna* is limited in its distribution to West Africa—since the embryos cannot be regarded as nocturnal. Probably this condition will be found to be much more extensively distributed. On the other hand we have described the embryos of *F. loa* as very similar to those of *F. nocturna* : but on closer study some points of difference may be noted in the disposition and number of the spots. Such a close resemblance indicates either that they are identical with *F. diurna* and that, therefore, *F. loa* is the parent form of *F. diurna*, or that, being very much alike in all other respects except in the matter of the spots as just mentioned, they are intended for a more or less similar life history in their intermediary hosts.

To sum up, although the weight of evidence is on the side of the identity of *F. nocturna* and *F. diurna*, there are many points which remain to be cleared up before the question can be settled. The *F. loa* has introduced a serious difficulty into the subject, and it appears to us that a solution of the mystery can only be obtained when the embryos in a pure case of *F. diurna* have been successfully and completely cultivated in their intermediary host—which is still to be discovered—to the final larval stage, and perhaps it may become necessary to perform experiments of infection of man by the use of infected intermediary hosts before a complete solution is procured.

APPENDIX

NOTES ON A COLLECTION OF MOSQUITOES FROM WEST AFRICA, AND DESCRIPTIONS OF NEW SPECIES

By F. V. THEOBALD, M.A., F.E.S., etc.

The collection of mosquitoes brought back by the members of the expedition from the West Coast of Africa contained twenty-six distinct species. Of these only five had been previously described. Thirteen of the new species are described in my Monograph of the *Culicidae* shortly to be published by the Trustees of the British Museum, and the remaining new ones here.

The collection includes the genera *Anopheles* Meigen (two species), *Mucidus* Theobald (one species), *Eretmapodites* Theobald (one species), *Stegomyia* Theobald (five species), *Culex* Linnaeus (nine species), *Panoplites* Theobald (one species), *Taeniorhynchus* Arribalzaga (modified) (two species), *Aedes* Meigen (one species), *Uranotaenia* Arribalzaga (three species).

The collection contained over two hundred and fifty specimens, including two midges (*Chironomidae*). Some of the types have been given me by the collectors for the British Museum.

Genus *Anopheles*. Meigen (1818)

(*Syst. Beschr. Eur. Zweifl. Ins.* p. 1-13, 1818)

I. *Anopheles costalis*. Loew

(*Berlin Ento. Zeitschr.* p. 55, 1866)

A number of this species taken at Bonny, Duke Town, Bugama, Bakana, Akwete Prison, s.s. Sobo (off Bakana), Lokoja, and at Old Calabar. They show considerable variation both in colour and size, but the costal markings and the spots on the femora remain distinct in all the specimens. Those from Old Calabar are considerably paler and somewhat smaller than those from Bonny. The specimens also show considerable variation in leg banding, it being almost absent in some, very distinct in others.

They were captured during the following months--April, in Duke Town; May and June, at Bonny; August, at Akwete; September, at Lokoja; in June, off Bakana; and June at Opobo.

II. *Anopheles barbirostris*. Van der Wulp var. *Africanus*

(*Leyden Museum Notes*, VI, p. 48)

Three dark ♀ *Anopheles* taken at Old Calabar in April are undoubtedly this species. They resemble in all structural respects the Asiatic form. The only difference to be noticed is that some pale scales are scattered over the wings, and there are no traces of leg banding. There is nothing upon which a new species could be founded, but they are certainly a local variety, and they look longer-legged than the Malay and Indian specimens I have seen.

The examination of the ♂ ungues *might* prove it to be quite distinct. I propose to call it variety *Africanus*; the variety based solely on the mottled wing scales.

black, apparently nude; palpi testaceous, with dark scales; antennae dark-brown, with narrow, pale bands, basal joint half testaceous, the inner half darker, base of the second joint testaceous, basal joint with a few small scales on the inner side, and minute curved hairs; proboscis deep brown; eyes black and golden.

Thorax deep chestnut-brown, with narrow, curved, deep-brown scales and ornamentation of similar bright golden ones, the latter most prevalent over and in front of the roots of the wings. Scutellum brownish, with flat, black scales on the middle lobe; narrower, rather curved, creamy ones on the lateral lobes, and with deep-brown border-bristles; metanotum brown; pleurae brown, with large patches of creamy scales.

Abdomen deep blackish-brown with narrow, white, basal bands, first abdominal segment rather ochraceous, covered with dusky-black scales and pale-brown hairs; posterior border-bristles chestnut brown, alternately long and short; venter mostly creamy white with narrow dark apical bands to the segments; the dorsal white bands form more or less white lateral spots.

Legs dark brown, pale at the base, femora grey ventrally; femora, tibiae, and metatarsi spiny; fore and mid ungues equal uniserrated, hind equal and simple.

Wings with the fork-cells rather short; scales brown; first submarginal cell very little longer and slightly narrower than the second posterior cell, their bases about level, stem of the former equal to about half the length of the cell, of the latter nearly two-thirds of its length; posterior cross-vein a little more than its own length distant from the mid cross-vein.

Halteres ochraceous, with pale scales over the knob, and dark ones on one side.

Length.—3 mm.

♂. Antennae black, with dense black plumes; palpi pale ochraceous, densely covered with black scales, the antepenultimate joint with two narrow pale rings; apical joint small, a little more than half the length of the penultimate joint, acuminate, penultimate joint wider than the apical, the antepenultimate expanding at the tip, the last two with long, brown hair tufts on one side, especially the penultimate, a few long hairs on the apex of the antepenultimate, and a few long black bristles on the apex of the last two joints; proboscis deep brown, almost black. Fore and mid ungues unequal, the larger uniserrated; hind ungues equal, small and simple. Fork-cells of wings small; the first submarginal cell shorter and considerably narrower than the second posterior, its stem nearly equal to the length of the cell; stem of the second posterior cell equal to the length of the cell.

Length.—4 mm.

Habitat.—Bonny.

Time of Capture.—May.

Observations.—Described from a series of ♀'s and a single ♂ in the collection of the Expedition. It is a clearly defined species, with banded abdomen and unbanded legs. The deep chestnut-brown thorax and grey and black head and unbanded legs separate it at a glance from all other African *Stegomyias* I have seen, except *S. nigeria*, from which it differs in thoracic ornamentation, the two parallel pale lines on the mesothorax of *S. nigeria* being absent.

VIII. *Stegomyia nigricephala.* Nov. sp.

(Fig. 3, Pl. I)

Head entirely black. Thorax dark-brown, with bronzy-brown scales. Abdomen black, with small, white, basal, lateral spots. Wings with dark-brown scales, and slightly tinged with brown. Legs dark-brown, unbanded.

♀. Head black (Fig. 3*b*, Pl. I), entirely covered with flat, black scales; clypeus, proboscis, and palpi black; antennae dark-brown; basal joint testaceous on one side, dark on the other; eyes golden.

Thorax black, with rather long, bronzy-brown curved scales, forming a dense matting over the black surface; over the roots of the wings numerous jet-black bristles; scutellum testaceous in the middle at the base, black at the apex, lateral lobes greyish-brown, mid lobe with flat black and grey scales and six (?) black border-bristles; metanotum blackish; pleurae very dark, with three large patches of white scales.

Abdomen (*c*) testaceous at the base, steely-black apically, covered with black scales, each segment with a small, basal, white, lateral spot; venter black, with basal white bands.

Legs dark blackish-brown, coxae and trochanters pale-brown; fore and mid (*d*) ungues equal, uniserrated, hind equal and simple.

Wings slightly tinged with brown; veins clothed with dark-brown scales; fork-cells small, the first sub-marginal cell a little longer but no narrower than the second posterior cell, its stem equal to about two-thirds of the length of the cell; stem of the second posterior as long as the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein.

Halteres with deep ochraceous stem and fuscous knob.

Length.—4·8 mm.

Habitat.—Bonny.

Time of Capture.—May.

Observations.—Described from a single ♀. The specimen was taken from a native hut. It can at once be told by the entire covering of black scales on the head and the rather long, curved, bronzy scales on the thorax and the unbanded abdomen.

Genus *Culex* L. (1735)

(*Syst. Nat.* 1735)

Palpi short in the ♀, long in the ♂, apical joint of latter usually acuminate, but sometimes clavate. Head clothed with narrow curved, upright-forked and broad flat lateral scales; scutellum covered with narrow curved scales; those on the thorax in three forms, narrow curved, narrow hair-like curved, and flat spindle shaped. Wings having the lateral vein scales linear, as a rule, and the first submarginal cell generally longer and narrower than the second posterior cell.

Eggs laid in rafts.

I have still retained several species in this genus which will have to be removed later.

IX. *Culex duttoni.* Nov. sp.

(Fig. 4, Pl. I)

Thorax dark-brown with golden-brown to golden narrow curved scales, with pale scaled areas in front, over the wings, two pale spots and pale scales in the middle of the back of the mesonotum, continuous with those over the wings. Abdomen with basal creamy-white bands. Legs with banding involving both sides of the joints.

♀. Head dark-brown with narrow creamy curved scales around the eyes, on the back of the occiput and in the middle, those between of a more golden-brown hue; the upright fork scales in front (forming a band around the head) bright brown, those behind creamy, at the sides of the head are a few small white flat scales; the fork scales are very numerous, there is also a row of bright-brown bristles projecting forwards over the eyes; clypeus black; palpi black scaled with a few

pure white ones up one side; proboscis deep blackish-brown, apex testaceous and with a dull testaceous band on the apical half; antennae deep-brown. Thorax black, covered rather densely with narrow golden-brown curved scales, and pale rather broader creamy ones arranged as follows:—around the front of the mesothorax, forming a narrow line, a more or less distinct spot on each side about the middle of the mesonotum, a long patch just over the roots of the wings, which bend round and pass up again on to the mesonotum, these latter are almost white; scutellum brown with narrow curved pale-golden scales, eight median golden-brown border-bristles, with some smaller fine pale golden ones over them; metanotum deep-brown; pleurae dark-brown with a few small patches of white scales.

Abdomen (*d*) deep-brown with basal dull creamy-white curved bands, and with more or less evident small lateral and basal pure white spots; border-bristles rather long, lateral ones also long.

Legs with the coxae and trochanters ochraceous; femora deep-brown, pale, almost white beneath, apex white; tibiae brown, with slightly paler base and apex, and with pale hairs; metatarsi with the apex pale banded, fore tarsi with the first and second joints apically and basally pale banded, the third basally banded, the fourth only showing a trace of basal banding. Mid tarsi the same as the fore; hind tarsi also very similar; ungues small, equal, and simple; hind metatarsi longer than the hind tibiae.

Wing with typical brown *Culex* scales; fork-cells rather long; first submarginal cell longer and narrower than the second posterior cell, its base nearer the base of the wing, its stem rather less than one-third the length of the cell; second posterior cell with its branches slightly contracted where they join the wing, its stem rather less than one-half the length of the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein. Halteres pale ochraceous.

Length.—4·8 to 5 mm.

♂. Palpi (*c*) dark-brown, with five white broken bands, last two joints with black hairs; apex of the antepenultimate also slightly hairy, apical joint acuminate; proboscis deep-brown, with an indistinct pale band; antennae dark-brown, with deep-brown plumes, faintly banded paler brown; basal joint deep ferruginous.

Abdomen narrow, the basal creamy-yellow bands prominent. The last segment with creamy-white scales in the middle; abdomen hairy. Legs banded much as in the ♀, but the last two tarsi seem to be unbanded; fore ungues unequal and uniserrated; hind equal, simple and small; wings with the fork-cells very small, first sub-marginal very little longer, not much narrower than the second posterior, its base nearer the apex of the wing than that of the second posterior cell, its stem slightly longer than the cell, posterior cross-vein about its own length distant from the mid cross-vein.

Length.—5 mm.

Habitat.—Duke Town.

Time of Capture.—April.

Observations.—Described from a series bred from larvae obtained at Canoes Creek, Duke Town. The thoracic ornamentation soon loses its characteristic appearance by denudation, the golden scales only remaining; the tarsal banding involving both sides of some of the joints and the faintly-banded proboscis should readily separate it from other African species. The banding on the abdomen in the male spreads out laterally in the sixth and seventh segments. The two, sometimes three, white bands on the antepenultimate joint of the male palpus are very characteristic, the most apical band being very wide.

X. *Culex decens.* Nov. sp.

(Fig. 5, Pl. I)

Thorax deep-brown to black with chestnut-brown scales; abdomen almost black with basal uniform white bands on the third to fifth segments, which widen out prominently on the sixth and seventh to form clear lateral spots. Legs dark-brown unbanded.

♀. Head almost black with small narrow curved creamy scales, and numerous dark upright-forked scales, quite black in some lights, the pale scales form a distinct line round the eyes; clypeus dark-brown; palpi deep-black; antennae dark-brown with black verticillate hairs and pale pubescence; proboscis deep bronzy-brown.

Thorax black, deep-brown in some lights, with very narrow curved bright chestnut-brown scales, rather paler in front, two dark median parallel lines show on the denuded surface; bristles deep-brown, especially long and thick over the roots of the wings; scutellum brown with very small narrow curved pale scales, seven bright-brown chaetae to the mid lobe; metanotum brown; pleurae ochraceous and slatey grey, with two patches of white scales, and an elongated patch just over the first two pair of legs.

Abdomen covered with deep blackish-brown scales, the first segment dull ochraceous with two median patches of dull-black scales and long pale hairs, the second to fifth segments with basal white bands, in the fifth the band spreads out a little laterally, on the sixth and seventh the band is rather broken in the middle but much expanded laterally, the eighth segment mainly white; border-bristles longest at the sides.

Legs brown, unbanded, coxae to base of femora pale, venter of femora grey, remainder deep-brown, femora, tibiae and metatarsi, especially of hind legs spiny; ungues small, equal, curved, simple.

Wings with the veins with typical brown *culex* scales; first long vein rather bent about half way along the wing; first submarginal cell longer and just slightly narrower than the second posterior cell, its base nearer the base of the wing, its stem equal to about one-third of its length; stem of the second posterior cell equal to about half the length of the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein; halteres with ochraceous stem and fuscous knob.

Length 5mm.

♂. Palpi all deep-brown to dull-black, just a trace of a narrow pale band near the base, the apical joint a little longer than the penultimate joint, acuminate, the two last joints with numerous blackish hairs, short and dense on the under surface only, a few also at the apex of the antepenultimate joint, the remainder with short, pale hairs all on the ventral surface, densely scaled below; the palpi are longer than the proboscis by the last joint and the apical third of the penultimate joint; proboscis dark-brown, apex testaceous; antennae grey, with deep-brown bands and brown plume-hairs.

Thorax as in the ♀; abdomen narrow, ornamented as in the ♀. Legs unbanded, traces of a pale knee spot; fore and mid ungues unequal, uniserrated; hind ungues small, equal.

Length.—4·5 mm.

Habitat.—Bonny.

Time of capture.—May.

Observations.—Described from a single ♂ and ♀ in the collection. The abdominal banding of the seventh and eighth segments expanding laterally, serves as a good means of identifying it at a glance.

XI. *Culex maculicrures.* (THEOBALD)
(*Mono. Culicidae,* Vol. I)

Four specimens (two ♂'s and two ♀'s) of this large brown species, bred from larvae taken at Bonny, and hatched during June. The ♀ measures between six and seven mm.; the thorax is dark-brown, with reddish-brown scales, and shows two prominent pale spots, with a pale line running from each backwards, and sometimes one or two pale and indistinct spots in front. The abdominal segments have narrow, apical, dull-yellow borders. The legs are brown and unbanded, but the femora and tibiae have a row of small yellow spots on one side.

This mosquito has a wide distribution in Africa, and Dr. BANCROFT has recently sent it from Australia (Queensland).

XII. *Culex metallicus.* THEOBALD
(*Mono. culicidae.* Vol. I)
(Fig. 14, Pl. III)

A number of this very distinct and pretty species, both ♂'s and ♀'s, taken during July, in the Bush opposite St. Stephen's Cathedral, Bonny.

It can at once be told by the thorax being silvery on the front half, brown on the posterior half, and by the more or less brilliant metallic violet abdomen, which is unbanded, as also are the legs, the femora being silvery at the base.

I have not seen this species from any other district in Africa, but I have the remains of a species very similar to it from Siam. It is only provisionally placed in *Culex*.

XIII. *Culex pruina.* Nov sp.
(Fig. 6, Pl. I, and Fig. 7, Pl. II)

Thorax covered with frosty-grey scales, with traces of two parallel darker lines; abdomen with the fifth to eighth segments with basal lateral white spots, almost forming bands, bases of the other segments slightly paler, in the ♂ with more or less distinct banding. Legs brown, unbanded.

♀. Head brown, clothed with hoary, narrow curved scales, and numerous ochraceous upright-forked ones; eyes black; clypeus, palpi, and proboscis deep-brown; antennae brown, basal joint paler.

Thorax shiny black, covered with thin, hair-like, curved hoary scales, and showing traces of two dark parallel bands on the denuded surface; scutellum with narrow curved hoary scales; metanotum testaceous and ochraceous; pleurae dark-brown above, ochraceous below. Abdomen (Fig. 6, Pl. I) dark-brown, almost black; the fifth to eighth segments with basal white lateral patches, which are most pronounced on the sixth, seventh, and eighth segments; the abdomen shows violet reflections; border-bristles pale. Legs brown, unbanded, ventral surface of the femora nearly white; ungues equal and simple.

Wings (Fig. 7*a*, Pl. II) with pale-brown, typical *Culex* scales; fork-cells rather long and narrow, the first submarginal longer, but no narrower than the second posterior cell, its base nearer the base of the wing than that of the latter; its stem about one-fourth the length of the cell. Stem of the second posterior cell about one-half the length of the cell; supernumerary cross-vein long and sloping, forming a very acute angle with the mid cross-vein; posterior cross-vein longer than the mid, and about one-and-a-half times its own length distant from it. Halteres ochraceous.

Length.—5 to 5·2 mm.

♂. Palpi ochraceous, covered with dark-brown scales, a small pale band near the base, the last two joints with dense black hairs, and also on one side of the apex of the antepenultimate joint; antennae banded, brown and grey, with deep flaxen-brown plumes; proboscis deep brown, apex testaceous. Abdomen narrow, expanding apically, the fourth and fifth segments with basal white bands, the sixth, seventh and eighth with pale bands expanded laterally, the ninth mostly white, moderately hairy. Fore and mid ungues unequal (Fig 7*b*, Pl. II), the larger one uniserrated, the smaller with a tooth near the base, hind equal and simple.

Length.—5 to 5·3 mm.

Time of capture.—August.

Habitat.—West Africa.

Observations.—Described from five specimens in this collection. A very distinct species with hoary scaled thorax, which has a dull golden tinge however in some lights, the banding of the abdomen and the form of the cross-veins are also characteristic.

XIV. *Culex invenustus.* Nov. sp.

(Fig. 8 and 9, Pl. II.)

Thorax dark-brown; abdomen black, unbanded and unspotted. Legs dark-brown, with pale-grey bases, fore and mid femora thick.

♀. Head (Fig. 8*b*, Pl. II) almost black, with narrow ochraceous-grey curved scales, blackish and brown, thin, upright-forked ones, flat white scales at the side, and a narrow white border round the eyes; eyes black; palpi short, dark-brown; proboscis rather short, dark-brown testaceous at the tip; antennae dark-brown, basal joint black, last two joints very hairy; clypeus black; thorax dark steely-black, covered with small, dull bronzy-brown, flat scales, forming a complete layer; when denuded the thorax shows three narrow parallel black lines; scutellum greyish-brown, with narrow curved pale scales and black border-bristles; metanotum dark-brown; pleurae ochraceous brown, slightly darker in front.

Abdomen deep blackish-brown, narrow, unbanded and unspotted; posterior border-bristles dull-brown; venter rather pale.

Legs unbanded, deep-brown, coxae pale, fore and mid femora (Fig 9, Pl. II) swollen, hind femora narrower, pale beneath, tibiae and metatarsi rather bristly; ungues small, much curved, equal and simple. Wings with brown scales of typical *Culex* form; fork-cells moderately long, the first sub-marginal considerably longer, but no narrower than the second posterior cell, its stem about one-fourth the length of the cell, its base nearer the base of the wing than that of the second posterior cell, stem of the latter, half the length of the cell; posterior cross-vein nearly twice its own length distant from the mid cross-vein.

Length.—3·5 mm.

Time of capture.—June.

Habitat.—Degama, West Africa.

Observations.—Described from a single perfect ♀. It can at once be distinguished by the general brown colour, unbanded and unspotted abdomen, and by the swollen fore and mid femora. It comes very near my *Culex longipes* in appearance.

The much swollen femora are probably of generic value, but I have only seen two specimens, both ♀'s, showing this character, and hence place them provisionally in *Culex*. *C. longipes mihi* comes from the Malay Peninsular.

XV. *Culex nebulosus.* Nov. sp.

(Fig. 10, Pl. II)

Head dark-brown with a pale border round the eyes. Thorax brown with tawny-brown scales. Abdomen dark-brown with traces of dull, grey apical lateral spots. Legs unbanded.

♂. Head dark-brown with narrow, curved, dull golden-brown scales, numerous brown, upright-forked ones, and a distinct white border round the eyes, and white scales at the sides; clypeus, proboscis, palpi, antennae brown, basal joint of the latter testaceous at the base; eyes black and golden.

Thorax shiny-black, covered densely with very narrow, curved, tawny-brown scales, and showing two darker parallel lines on the denuded surface, numerous golden-brown and dark-brown bristles over the roots of the wings; scutellum brown with very narrow, almost hair-like, pale scales, seven bristles to the mid lobe; metanotum dark chestnut-brown; pleurae brown and ochraceous with scanty flat white scales.

Abdomen deep-brown, unbanded, with dull violet reflections, indistinct apical, creamy-white lateral spots (Fig. 10*c*, Pl. II); venter grey and brown.

Legs brown, unbanded; coxae and trochanters ochraceous, the former with dull white scales; femora dull, pale ochraceous beneath.

Wings (Fig. 10*a*, Pl. II), with brown scales of typical *Culex* form; first submarginal cell considerably longer and a little narrower than the second posterior cell, its stem less than one-third the length of the cell; stem of the second posterior equal to about half the length of the cell; posterior cross-vein considerably longer than the mid cross-vein, about its own length distant from it.

Halteres with slightly fuscous knob and ochraceous stem.

Length.—3 to 3·5 mm.

Time of capture.—April, August, September.

Habitat.—Old Calabar.

Observations.—Described from six specimens. A rather obscure species, with traces more or less distinct of pale, apical, lateral, abdominal spots, and rather marked cephalic ornamentation.

XVI. *Culex fatigans* Wied (1828)

(*Ausseurop. Zweiflug Ins.* p. 10)

This common mosquito also occurs in West Africa, but is only represented in the collection by a single ♀. It does not seem common, however, in this part of Africa judging from the collections I have received from Bonny and the neighbourhood, but, perhaps, owing to its commonness, it has not been collected. Like *S. fasciatus* Fab. its distribution is very wide, and it is one of the most troublesome species, biting chiefly at night and acting as one of the *Filaria* carrying hosts.

It closely resembles *Culex pipiens* L. of Europe and North America, but it can always be told by the stem of the first submarginal cell being relatively much longer than in *C. pipiens.* The stem in *C. pipiens* is never less than one-fifth the length of the cell, in *C. fatigans*, it is always more, often only one-third the length.

XVII. *Culex rima.* Nov. sp.

(Fig. 11, Pl. II)

Thorax deep-brown. Abdomen deep-brown, with metallic-bronze and violet reflections, white, apical, lateral spots and grey venter. Legs deep-brown, unbanded. Wings with rather broad scales like *C. atratus*, THEO. Ungues small, curved, equal, and simple.

♀. Head dark-brown, with narrow, curved, dull-grey scales and numerous short, upright, black ones; clypeus (*b*) black, *with a transverse sulcus*; antennae brown, with reddish-brown basal joint; proboscis black, testaceous at the apex; palpi rather thick, black.

Thorax deep-brown, with very minute, narrow curved, dull-brown scales and long black, backwardly-projecting bristles; scutellum deep chestnut-brown in the middle, greyish apically, with narrow dull-brown curved scales and black border-bristles; metanotum deep-brown; pleurae greyish or greyish-brown.

Abdomen bronzy-black, with deep bronzy-green and deep-violet reflections when held in different lights, the four posterior segments with *four distinct, white, apical spots*; posterior border-bristles dull-brown, short; apex pubescent. Legs deep-brown; the coxae very pallid, and also the venter of the femora; the metatarsi and tarsi with somewhat dull, ochraceous reflections ventrally. Ungues small, equal, and simple.

Wings (Fig. 11*a*, Pl. II) densely scaled towards their apices with rather short, thick, brown scales (a^1) (as in *C. atratus* THEO.); fork-cells rather short, first submarginal cell longer and narrower than the second posterior cell, their bases not nearly level, that of the former, nearer the base of the wing; stem of the first submarginal equal to about half the length of the cell; stem of the second posterior as long as the cell; posterior cross-vein slightly curved in the middle, nearly three times its own length distant from the mid cross-vein; fringe brown, very dark at the apex of the wing.

Halteres with ochraceous stem and fuscous knob.

Length.—2·8 mm.

Habitat.—Old Calabar.

Time of capture.—April.

Observations.—Described from three ♀'s. A small species with very distinct abdominal ornamentation. In two specimens the thorax is paler brown. It is closely related to the little black *Culex* I call *Culex atratus*, common in Jamaica. The peculiar wing scales and general facies of these two species will necessitate their removal from *Culex*, but I am waiting for more material as I have only received one damaged ♂ (*C. atratus*) of this group.

XVIII. *Culex invidiosus.* Nov. sp.

(Fig. 12, Pl. II)

Head deep-brown with greyish sheen, seen in some lights; thorax deep chestnut-brown; abdomen blackish-brown, unbanded and unspotted; pleurae paler brown; legs deep-brown, coxae and bases of femora pale.

♀. Head deep-brown, almost black, covered with dull ochraceous grey narrow curved scales over the occiput, black upright-forked ones, and small flat dull white lateral ones, a narrow, rather indistinct grey border round the eyes; clypeus deep chestnut-brown; proboscis deep blackish-brown; palpi short, densely black scaled; antennae brown, basal joint testaceous in the centre.

Thorax brown, with narrow curved dull golden-brown scales, and black bristles; scutellum rather shiny, rich brown, with narrow curved dull-grey and brown scales, six or seven bristles to the mid lobe, and four each to the lateral lobes; metanotum deep-brown; pleurae pale ochraceous brown.

Abdomen deep-brown, with slight deep-violet reflections; narrow; border-bristles short and pale, apex testaceous, rather hairy; venter brown, hairy, testaceous at the base; the scales at the sides, in some lights under the microscope, have a dull violet-grey hue.

Legs deep-brown, with violet reflections, coxae pale ochraceous, with a number of pale hairs; venter of femora pale ochraceous, tibiae and bases of the metatarsi with a few bristles; ungues small, equal, and simple.

Wings with typical brown *Culex* scales, first submarginal cell longer and a little narrower than the second posterior cell, its stem is about one-third the length of the cell, its base nearer the base of the wing than that of the second posterior, stem of the latter about two-thirds the length of the cell; mid cross-vein long; posterior cross-vein not quite twice its own length distant from the mid.

Halteres with ochraceous stem and fuscous knob.

Length.—3·2 mm.

Habitat.—Bonny.

Time of capture.—May.

Observations.—Described from a single ♀. I do not know any species at all resembling it, yet there are no very distinctive characters. The unbanded legs and abdomen, and its general brown color, when roughly examined, make it resemble *Aedes nigra*, but it can at once be told from it by the head and wing scales, which are of typical *Culex* form.

Another ♀ differs considerably in colour, but I can detect no structural difference. It is much paler, of a general ochraceous tint, due to denudation of the scales. The thorax is paler brown with two pale median parallel stripes in front, separated by a darker line, and the scutellum has seven mid bristles, and the venter of the abdomen is paler and grey scaled. Venation, scales, ungues, etc., are similar, and it was taken in the same place and date as the type. I fancy one is full of ova, the other dark with blood.

GENUS *Panoplites*. THEOBALD (1901)

(*Mono. Culicidae*, Vol. II)

This genus differs from *Culex* chiefly in the peculiar formation of the wing scales, which are broad and asymmetrical squamae, concave at their free extremity (Fig. 13, Pl. II). This character will suffice to identify the genus. The eggs are laid singly, and taper to a point at one end. Many of the species are vicious biters, and chiefly occur along river banks. The African species here mentioned acts as the *Filaria* host.

XIX. *Panoplites africanus*. THEOBALD

(*Mono. Culicidae*, Vol. II)

Quite a number of this species occur in the collection from Asaba taken in June, July, and August. The thickly scaled wings will at once separate it from other *Culices* occurring in

the neighbourhood. The legs are broadly basally banded white, and the femora and tibiae more or less mottled ; the general colour is rich brown, the abdomen being deeper brown, with apical white patches of lateral scales, and similar ochraceous basal ones. Some specimens show apical ochraceous bands ; the scales are not evenly disposed and give the abdomen a slight ragged appearance. The thorax shows characteristic ornamentation under the microscope, the greater surface being covered with golden-brown scales, with lines and patches of silvery-grey scales. The specimens collected at Asaba differ in no respects from those in the other parts of West and Central Africa.

GENUS *Taeniorhynchus* ARRIBALZAGA (1891) (Modified F.V.T.)

(*Dipt. Argentina*, p. 47, and *Mono. Culicidae*, Vol. II)

Separated from *Culex* by Arribalzaga chiefly on account of the palpal structure and ungues and banded rostrum. His genus, however, contains three totally diverse species. I have, therefore, remodelled it upon his *T. fasciolatus* (Vide *Mono. Culicidae*).

The only feature I need point out here is that the wings are always covered along the veins with thick elongated scales, giving the wings a densely scaled appearance, but quite different to *Panoplites* in form. I know nothing of the life-history of any of the species in this genus.

XX. *Taeniorhynchus aurites*. THEOBALD

(*Mono. Culicidae*, Vol. II)

Eight or nine ♀'s of this pretty golden-yellow gnat were taken at Bonny and Ogugumanga. One bears on the label 'Taken in the bush opposite St. Stephen's Cathedral.' They were captured in May, June, and July. It can be told from the other yellow African mosquito by the thorax being honey-yellow and unadorned ; the hind legs have apical dark bands to the metatarsi and tarsi, and the wings brilliant orange-yellow.

XXI. *Taeniorhynchus annettii*. THEOBALD

(*Mono. Culicidae*, Vol. II)

A ♂ and eight ♀'s taken at Old Calabar at the Vice-Consulate in April, and at Bonny.

It resembles *T. aurites* but the sixth vein is dark scaled ; there is darker thoracic ornamentation and apical dark banding to the fore and mid legs, more or less distinct ; the abdomen has apical deep-violet bands.

GENUS *Aedes*. MEIGEN (1818)

(*Syst. Beschr.* Vol. I, p. 13, 1818)

Palpi short in both ♂ and ♀. Head clothed with both flat and narrow-curved scales, the flat scales predominating ; scutellum with narrow-curved scales only. Fork-cells of the wings moderately long ; scales on the wings very similar to *Culex*, there being always long, thin, lateral scales to the veins, which are not seen in other genera of the *Aedeomyina*.

Two species occur in the genus in Africa.

XXII. *Aedes nigra.* THEOBALD

(*Mono. Culicidae,* Vol. II)

Five ♀'s and one ♂ of this small dark *Aedes* only about 2mm. long. Taken at Old Calabar in April. It can readily be told by its black appearance, unbanded legs, abdomen, and absence of thoracic ornamentation. From the *Uranotaenia* it can at once be distinguished by the relative greater length of the fork-cells.

GENUS *Uranotaenia.* ARRIBALZAGA (1891)

(*Dipt. Argentina,* p. 63, 1891)

Palpi short in the ♂ and ♀ as in *Aedes,* but the fork-cells are very small, especially the first submarginal fork-cell. There are always flat scales, usually brilliant in places on the mesonotum and on the scutellum, and the head is entirely covered with flat scales. Many of the species bite severely. The larvae are often brilliantly coloured with red, blue, and green, and seem to be intermediate between *Anopheles* and *Culex* in structure.

XXIII. *Uranotaenia domestica.* THEOBALD

(*Mono. Culicidae,* Vol. II)

Two specimens of this beautiful *Uranotaenia* taken at Old Calabar at the Vice-Consulate, in April. One badly damaged.

It can easily be identified by the bright, chestnut-brown thorax, with a small, silvery spot on each side in front, another on the roots of the wings, a bright, silver-scaled scutellum; the abdomen is almost black, with white lateral spots, and the legs are black with a white spot at the apex of the tibiae and femora, and a silvery band near the apex of the hind femora.

Length.—4 mm.

XXIV. *Uranotaenia annulata.* THEOBALD

(*Mono. Culicidae* Vol. II)

Three ♀'s and three ♂'s taken at Bonny in May. A very marked little *Uranotaenia,* with chestnut-brown mesothorax and sharply contrasted pale creamy pleurae and head, the latter having a dark median line. The abdomen is brown, and has apical grey or white bands. Legs brown; the hind ones with the metatarsi and first two tarsi with apical white bands, and the last two joints pure white.

XXV. *Uranotaenia caeruleocephala.* THEOBALD

(*Mono. Culicidae,* Vol. II)

Eight ♀'s taken in April at Old Calabar. It is a beautiful little deep-brown species, easily identified by its sky-blue head. The legs and abdomen are unbanded. On the thorax may be seen a line of white scales at the sides, just in front of the wings.

Length.—2·5 mm.

PLATES TO APPENDIX

PLATE I

FIG. 1 *Eretmapodites quinquevittata.*—Fore and mid ungues of ♂, and fore ungues of ♀: ♂ palpus and apex of ♂ hind legs.

FIG. 2. *Stegomyia irritans.* Nov. Sp.—♂ palpus and cephalic ornamentation.

FIG. 3. *Stegomyia nigricephala.* Nov. sp.—*a*, wing of ♀; *b*, head; *c*, abdominal ornamentation; *d*, fore ungues of ♀.

FIG. 4. *Culex duttoni.* Nov. Sp.—*a*, thorax of ♀; *b*, markings on denuded thorax; *c*, ♂ palpus; *d*, abdominal ornamentation.

FIG. 5. *Culex decens.* Nov. sp.—♂ palpus.

FIG. 6. *Culex pruina.* Nov. sp.—♂ and ♀ abdominal ornamentation.

PLATE II

FIG. 7. *Culex pruina.* Nov. sp.—*a*, wing of ♀; *b*, fore ungues of ♂

FIG. 8. *Culex invenustus.* Nov. sp. *a*, wing of ♀; *b*, head ornamentation.

FIG. 9. *Culex invenustus.* Nov. sp. —Fore leg to first tarsal joint.

FIG. 10. *Culex nebulosus.* Nov. sp.—*a*, wing of ♂; *b*, cephalic ornamentation; *c*, abdominal ornamentation.

FIG. 11. *Culex rima.* Nov. sp. *a*, wing of ♀; a^1, apical wing scales; a^2, basal scales; *b*, clypeus.

FIG. 12. *Culex invidiosus.* Nov. sp.—*a*, scutellar bristles; *b*, wing of ♀

FIG. 13. Wing scales of *Panoplites.*

PLATE III

FIG. 14. *Culex metallicus.* THEOBALD.—*a*, thoracic ornamentation; a^1 and a^2 enlarged scales; *b*, ♂ palpus; *c*, fore and hind ♀ ungues; *d*, apex of antenna; *e*, wing fringe; *f*, ♂ genitalia; *g*, wing scales; *i*, another form of wing scales; *h*, fore and hind ♂ ungues.

Fig. 1

Fig. 2

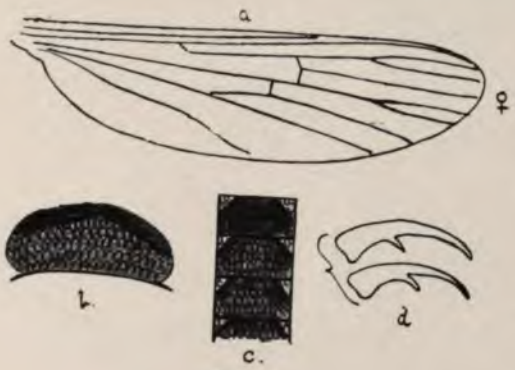


Fig. 3

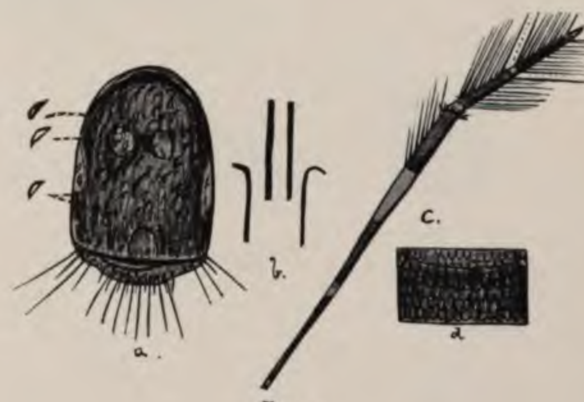


Fig. 4

Fig. 5

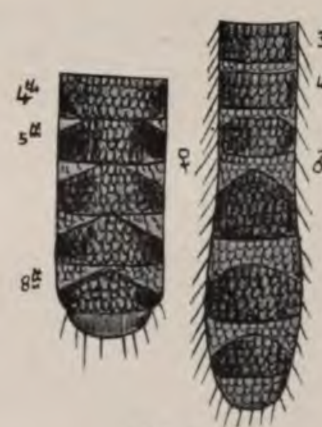


Fig. 6

PLATE II OF APPENDIX

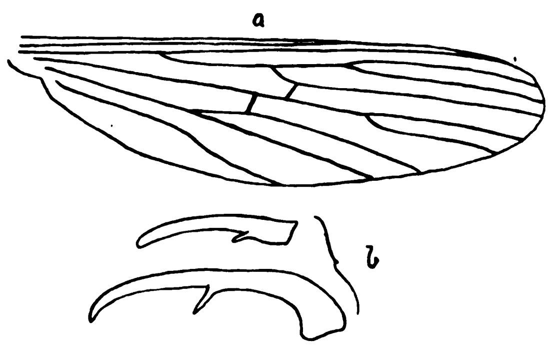
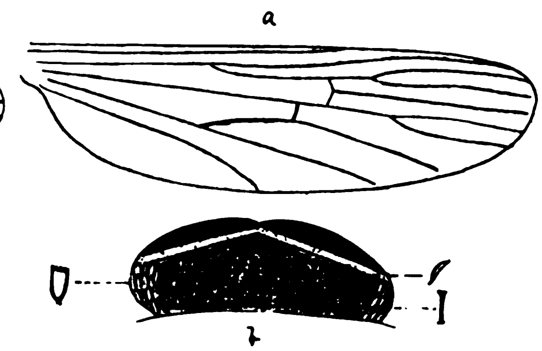


FIG. 7



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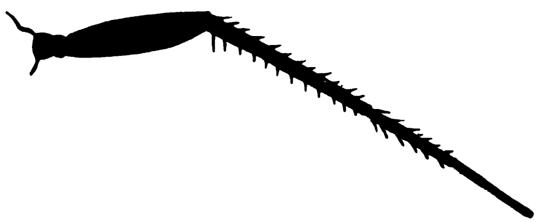


FIG. 9

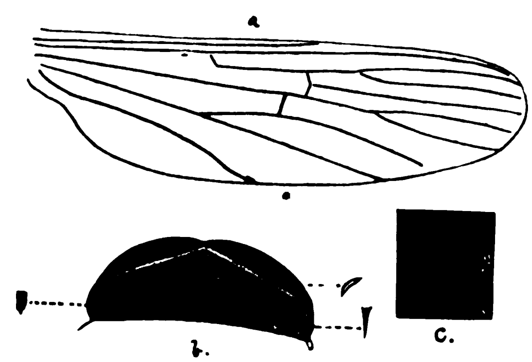


FIG. 10

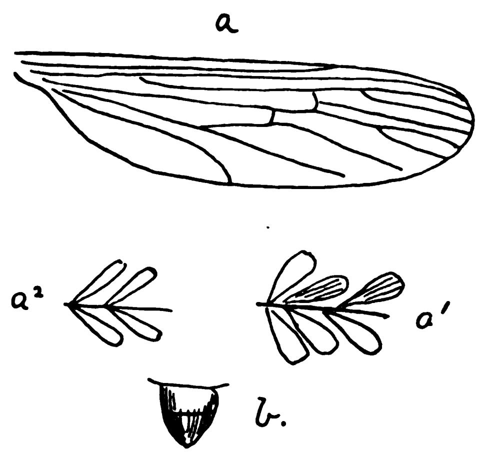


FIG. 11

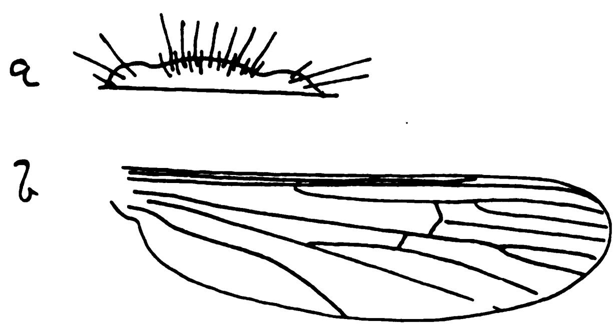


FIG. 12

FIG. 13

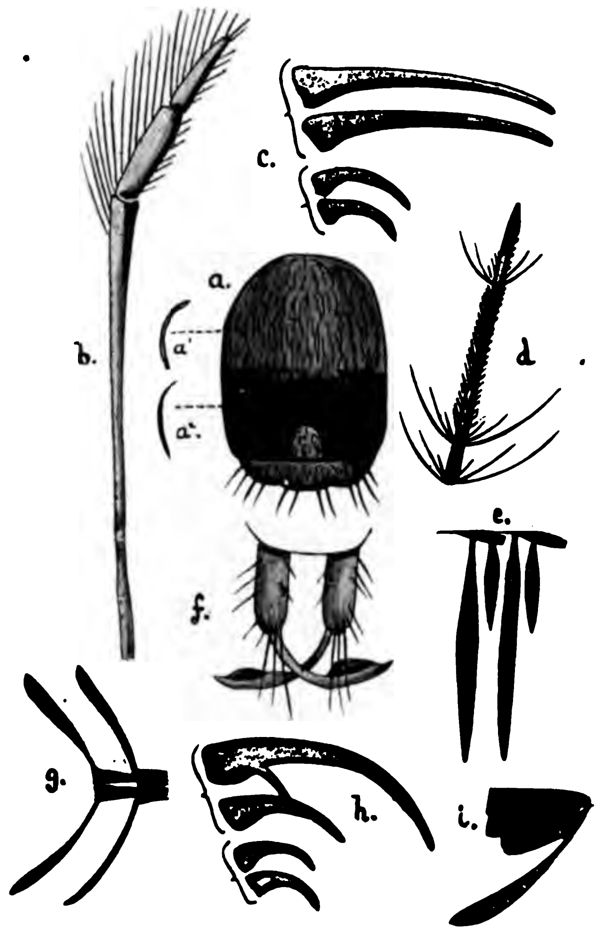


Fig. 14

DESCRIPTION OF PLATES

PLATE I

FIG. 1. *Filaria cypseli.* Nov. sp. ♂ and ♀: natural size.

FIG. 2. *F. cypseli.* Nov. sp. Head end of ♀. *a*, alimentary tract; *b*, oesophagus; *c*, opposite vaginal orifice; *d*, uterus full of ova; *e*, vagina. The nerve collar is indicated by the dark band across the anterior portion of the oesophagus.

FIG. 3. *F. cypseli.* Tail end of ♀. *a*, position of anus; *b*, uterus.

FIG. 4. *F. cypseli.* Head end of ♂. *a*, oesophagus; *b*, intestine.

FIG. 5. *F. cypseli.* Tail end of ♂. The worm has been ruptured near the extreme end, where the body contents are extruded. *a*, alimentary tract; *b*, opposite position of anal orifice; *c*, spermatic tube; *d*, spicules.

PLATE II

FIG. 1. *Filaria spiralis avium.* Nov. sp. ♂ and ♀: natural size.

FIG. 2. *F. spiralis avium.* Anterior end of ♀, and portion of first spiral. *a*, alimentary tract; *b*, oesophagus; *c*, opposite the position of vaginal orifice; *d*, uterus; *e*, vagina.

FIG. 3. *F. spiralis avium.* Posterior end of ♀, and portion of last spiral. *a*, alimentary canal; *b*, opposite the anal orifice, which is seen to be surrounded by five delicate lips, giving a rosette appearance; *c*, the lateral ridge as seen in the concavities of the coils; *d*, uterus, full of ova.

FIG. 4. *F. spiralis avium.* Posterior end of the ♀, side view; *a*, alimentary canal; *b*, opposite the position of anal orifice: seen here as a baying in the cuticle; *c*, the lateral cuticular ridge as seen on the convexities of the spirals; *d*, uterus.

FIG. 5. *F. spiralis avium.* The embryo: stained specimen of the blood (× 250). The dark spots round the worm are the nuclei of the red corpuscles. The sheath of the worm is distinctly shewn both at the head and tail ends of the worm.

PLATE III

FIG. 1. *Filaria spiralis avium.* Tail end of ♂ shewing its shape; *a*, opposite the position of the anal orifice; *b*, alimentary tract; *c*, spermatic tube.

FIG. 2. *F. spiralis avium.* Shews the spicular arrangement of the male. The two spicules are seen extruded through the wide crater-like anal orifice, situated on a low papilla. Behind is indicated the lateral cuticular flange which here comes to the ventral surface, to form with the one of the other side a sort of hollow cone at the bottom of which is the anal orifice.

PLATE IV

FIG 1. *Filaria fusiformis avium.* Nov. sp. ♂ and ♀: natural size.

FIG 2. *F. fusiformis avium.* Anterior end of ♀ shewing its shape, and *a*, the position of the vaginal orifice.

FIG 3. *F. fusiformis avium.* Posterior end of ♀ shewing its shape; *a*, alimentary tube; *b*, near the termination of the uterine tube.

FIG. 4. *F. fusiformis avium.* The embryos: a specimen of stained blood shewing the embryos inside, partly and completely out of their sheaths, also an empty sheath. The position of some of the 'spots' is also seen (× 350).

PLATE V

FIG. 1. *Filaria spiralis major avium.* Nov. sp. ♀ and ♂: natural size.

FIG. 2. *F. spiralis major avium.* Anterior end of ♀. *a*, opposite the anal orifice; *b*, uterus; *c*, vagina.

FIG. 3. *F. spiralis major avium.* Tail end of ♀. *a*, opposite the anal orifice. The cuticular knobs are well seen on the convexities of the spirals.

FIG. 4. *F. spiralis major avium.* Anterior end of ♂. *a*, oesophagus; *b*, alimentary canal; *c*, spermatic tube.

FIG. 5. *F. spiralis major avium.* The strongly incurved tail of the ♂. *a*, opposite the position of the anal orifice and spicules.

PLATE VI

FIG. 1. *Filaria spiralis major avium.* The embryo in a specimen of stained blood, shews the position of some of the 'spots' and the characteristic wire nail shaped posterior end (× 250).

FIG. 2. *F. shekletonii.* Nov. sp. The embryo in a specimen of stained blood. The position and characters of the 'spots' are well marked, as well as the sharply pointed tail (× 250).

FIG. 3. *F. shekletonii.* ♀: natural size.

FIG. 4. *F. shekletonii.* Head end of ♀. *a*, oesophagus; *b*, the alimentary tract; *c*, opposite vaginal orifice; *d*, uterus.

FIG. 5. *F. shekletonii.* The posterior end of ♀. *a*, intestine; *b*, opposite anal orifice; *c*, uterus.

PLATE VII

FIG. 1. *Filaria falciformis.* Nov. sp. ♂ and ♀: natural size.

FIG. 2. *F. falciformis.* Nov. sp. Head end of ♀. *a*, oesophagus; *b*, intestinal canal; *c*, opposite vaginal orifice; *d*, vagina; *e*, uterus.

FIG. 3. *F. falciformis.* Tail end of ♀. *a*, position of anal orifice; *b*, ovary; *c*, uterus; *d*, the corrugated cuticle.

FIG. 4. *F. falciformis.* Head end of ♂. *a*, oesophagus; *b*, intestine; *c*, opposite position of nerve collar crossing the oesophagus.

FIG. 5. *F. falciformis.* Tail end of ♂ shewing the spicules *a* and *b* extruded through the wide anal orifice; *c*, papillae; *d*, base of spicules.

PLATE VIII

FIG. 1. *Filaria falciformis.* Tail end of ♂ shewing spicular arrangement not extruded; numerous spermatozoa are seen.

FIG. 2. *F. falciformis.* The embryo in a specimen of stained blood; shews the characteristic 'spot' (× 250).

FIG. 3. *F. bibulbosa.* Nov. sp. ♂ and ♀ : natural size.

FIG. 4. *F. bibulbosa.* Head end of ♀. *a*, intestinal tract; *b*, oesophagus; *c*, opposite vaginal orifice; *d*, uterus; *e*, vagina.

FIG. 5. *F. bibulbosa.* Tail end of ♀ ; *a*, opposite anal orifice ; *b*, intestine ; *c*, distal end of ovary.

PLATE IX

FIG. 1. *Filaria bibulbosa.* Head end of ♂ ; *a*, intestinal canal ; *b*, oesophagus ; *c*, spermatic tube.

FIG. 2. *F. bibulbosa.* Tail end of ♂ ; note the single extruded spicule.

FIG. 3. *F. bibulbosa.* The embryos in stained blood. Specimen shewing their comma-shape and 'spots' (× 250).

PLATE X

FIG. 1. *Filaria capsulata.* Nov. sp. ♂ and ♀ and cyst containing worms : natural size.

FIG. 2. *F. capsulata.* The cyst with ♀ worm enclosed were highly magnified. The ♂ had been removed.

FIG. 3. *F. capsulata.* Head end and portion of the body of ♀ ; *a*, oesophagus ; *b*, intestine ; *c*, opposite vaginal orifice; *d*, vagina ; *e*, uterine horn.

FIG. 4. *F. capsulata.* Tail end of ♀ ; *a*, intestine ; *b*, opposite anal orifice.

FIG. 5. *F. capsulata.* The embryo in stained blood preparation (× 250).

PLATE XI

FIG. 1. *Filaria capsulata.* The ♂ complete. *a*, oesophagus; *b*, intestine; *c*, spermatic tube; *d*, tail end; *e*, head end.

FIG. 2. *F. capsulata.* Tail end of ♂. *a*, opposite anus and single partly extruded spicule; *b*, intestine; *c*, head end of ♂ ; *d*, head end of ♀.

FIG. 3. *F. phoenicopteri.* Nov. sp. ♂ : natural size.

FIG. 4. *F. phoenicopteri.* Head end of ♂. *a*, oesophagus; *b*, opposite oral orifice.

FIG. 5. *F. phoenicopteri.* Tail end of ♂ shewing single spicule extruded. *a*, intestine.

PLATE XII

FIG. 1. *Filaria serpentiformis.* Nov. sp. The embryo in stained blood preparation (× 250).

FIG. 2. *F. opobensis.* Nov. sp. The embryo in stained blood preparation (× 350).

FIG. 3. *F. calabarensis.* Nov. sp. The embryo in stained blood preparation (× 250).

PLATE XIII

FIG. 1. *Filaria cypseli.* Embryo with sheath—fresh specimen (× 550).

FIG. 2. *F. spiralis avium.* Embryo with sheath—fresh specimen (× 550).

FIG. 3. *F. fusiformis avium.* Embryo, and its head ending showing prepuce, papilla, and spine protruded and retracted (× 550).

FIG. 4. *F. spiralis major.* Embryo and sheath (× 550).

FIG. 5. *F. falciformis.* Embryo (× 550).

FIG. 6. *F. bibulbosa.* Embryo (× 550).

PLATE XIV

FIG. 7. *Filaria capsulata.* Embryo (× 550).

FIG. 8. *F. serpentiformis.* Embryo (× 550).

Plate XV

Fig. 1. Transverse section of proboscis of the female *Anopheles costalis* near its tip (× 460). *lr-ep*, labrum-epipharynx; the two portions are shewn separated by a thin red transverse band; *h*, hypopharynx, with salivary canal at its centre; *m*, mandible; *mx*, maxilla; *lb*, labella; *t*, tip of labium; *fh*, superior region of inner surface of labella from which arises a feltwork of fine hairs; *ch*, inferior region of inner surface from which coarse hairs arise; *r*, a ridge of thickened chitin on the middle region of the inner surface, which above at its base enters into the articulation of the labella and labium.

Fig. 2. Transverse section of proboscis at the level of the labella joints (× 460). *lr-ep*, *h*, *m*, *mx*, as in fig. 1; *mx.p*, maxillary palp; *l*, lateral pear-shaped area at extremity of labella; *ln*, nerve to the labella; *a*, chitinous articulating surface of the labium; *k*, triangular area, occupied by a loose delicate membrane hanging from beneath the portion of the upper chitinous surface of the labium, which is prolonged to the extreme tip of the proboscis. In the section, the cut edge of the membrane is shewn as an irregular line. In this figure the labrum is not represented.

Fig. 3. Transverse section about the level of the middle of the proboscis (× 460). *lr-ep*, *h*, *m*, *mx*, *mx.p.* as in figs. 1 and 2; *l*, labium; *l.tr*, trachea to the labium; *l.n*, nerve to the labium; *r*, lateral chitinous ridge of the labium; *l.m*, labellar muscles. In this figure the labrum is not represented.

Plate XVI

Fig. 1. Transverse section at the base of the proboscis of the female *Anopheles costalis* (× 460). *lr*, labrum; *ep*, epipharynx; *ep.r*, lateral supporting chitinous ridge of the epipharynx containing core of chitin forming cells; *h*, hypopharynx with salivary gutter; *m*, mandible; *mx*, maxilla; *mx.p*, maxillary palp; *o'*, a concave region on the inner surface of the maxillary palp, against which the mandible fits, indicating the relation of its origin; *o''*, a similar region for the maxilla; *p.m*, muscle of the maxillary palp; *l*, labium, note the shape at this level as compared with sections, plate xv, fig. 3; *r* lateral chitinous ridge of labium; *l.tr*, labial trachea; *ln*, labial nerve.

Fig. 2. Transverse section of proboscis just before the separation of the various mouth parts from each other (× 400). *c*, clypeus; *f*, upper posterior angle of the fulcrum; *lr*, proximal extremity of labrum, note the cubical cells; *lr.p*, chitinous prolongation of the labium within the clypeus; *em*, epipharyngeal muscle; *ep*, epipharynx; *h*, hypopharynx, the apex of the salivary receptacle is seen below, supported by two lateral chitinous bars; *m*, mandible; *mx*, maxilla, note its sickle shape; *mx.p*, maxillary palp; *pn*, nerve to maxillary palp; *pm*, muscle to the maxillary palp; *l*, labium; *ln*, labial nerve; *ltr*, labial trachea; note the line of cleavage of the labium from the other mouth parts.

Plate XVII

Fig. 1. Transverse section of the head of *Anopheles costalis*, at the level of the middle of the ascending portion of the pharynx (× 360). *p*, ascending portion of the pharynx; *pd*, middle membranous portion of the upper wall of the pharynx, consisting of a layer of low cubical epithelium; *pv*, lower chitinous plate of the pharynx; *pm*, pharyngeal muscle; *lbr.m*, fan-shaped labral muscle; *sd*, common salivary duct;

rm, muscle to the salivary receptacle; *mx.p'*, intercranial maxillary process; *zm*, muscle attaching maxillary process to the occipital region of head; *lm'*, muscle to base of labium; *ln*, nerve to the proboscis; *ltr*, trachea to the proboscis; *mm*, muscle to the base of the mandible.

Plate XVIII

FIG. 1. Semi-diagramatic sagittal section through the head and proboscis of the female *Anopheles costalis* (× 200). *lbr*, labrum; *ep*, epipharynx; *h*, hypopharynx; *l*, labium; *p'* ascending portion to the pharynx; *p''*, horizontal portion; *n*, nerve to the proboscis; *oe*, oesophagus; *tr*, trachea to the proboscis; *s.r*, salivary receptacle; *s.d*, common salivary duct; *f.m*, muscle to the salivary receptacle; *x*, chitinous ridge or under surface of ventral wall of the first part of the pharynx, from which *(f.m)* the muscle to the salivary receptacle arises; *c*, clypeus; *p.m*, pharyngeal muscle; *lbr.m*, labral muscle inserted into the prolongation of the labrum; *e.m*, epipharyngeal muscle arising from the fulcrum; *a*, commencement of the labrum: below this on the upper wall of the pharynx are the 'taste papillae'; *f*, fulcrum; *s.o.g*, supra-oesophageal ganglion; *i.o.g*, infra-oesophageal ganglion; *s.o*, specialized hairs; *mx.p*, intercranial maxillary process; *v.c*, ventral commissure; *d.v*, dorsal vessel; *d.t*, main trachea to the head; *e*, eye.

FIG. 2. Transverse section at the level of the junction of the first and second part of the pharynx shewing the group of specialized hairs (× 530). *p*, pharynx; *z*, that part of the exoskeleton which is folded in beneath the eyes.

Plate XIX

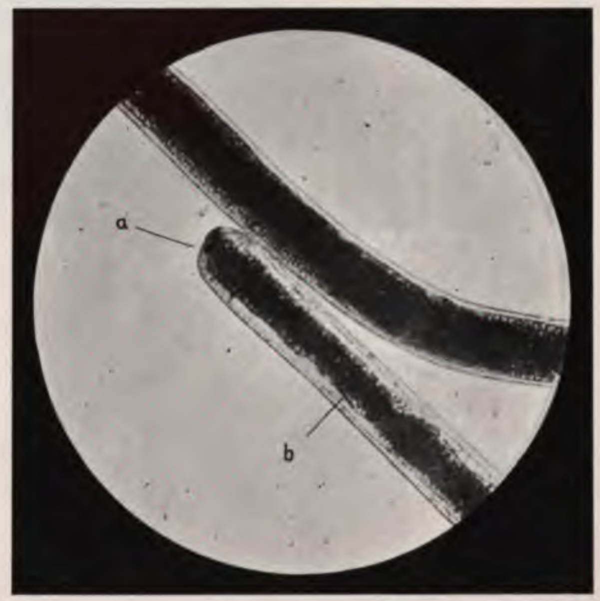
FIG. 1. Semi-diagramatic longitudinal horizontal section of the head and proboscis of the female *Anopheles costalis* (× 180). *bm*, muscle to the pumping organ, the middle membranous portion of the pharynx; *zm*, muscle attaching the maxillary process to the occipital region of the skull; *ltr*, trachea and nerve to the proboscis; *lm'*, muscle to the base of the labium, arising from the under surface of the maxillary process; *rm*, muscle to the salivary receptacle; *sr*, salivary receptacle and duct; *s*, V-shaped opening of salivary receptacle; *h*, hypopharynx, the salivary gutter runs along its centre; *mx.p*, maxilliary palp; *lm*, origin of labellar muscle; *mx.p'*, intercranial process of the maxilla; *e*, eye,

FIG. 2. Section of the distal end of the labium and labellae (× 410). *lm*, labellar muscle; *lm'*, longitudinal tendon of labellar muscle; *r*, lateral chitinous ridge of the labium, from which the labellar muscles arises; *a*, chitinous process at the base of the labella into which the long tendon of the labellar muscle is inserted; *lb*, labella; *ln*, the termination of the labellar nerve; *g*, ganglionic structure in the interior of the labella, shewing the fibres of the labellar nerve ramifying over its surface; *ch*, coarse hairs projecting downwards between the labellae and arising from their inner surface.

FIG. 3. Drawing of a cleared specimen of the distal end of the labium and of the labellae of the female *Anopheles costalis* (× 390). *lb*, labella; *l*, labium; *g*, groove on the upper surface of the labium in which the stylets are enclosed; *a*, the labellae articulation, observe the angle for the insertion of the long tendon of the labellar muscle; *r*, lateral chitinous ridge of labium; *tl*, tip of the labium.

Fig. 1

Fig. 2



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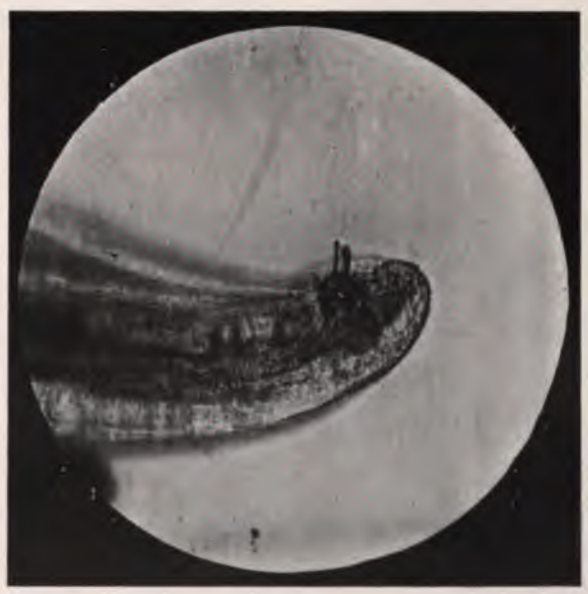

Fig. 2

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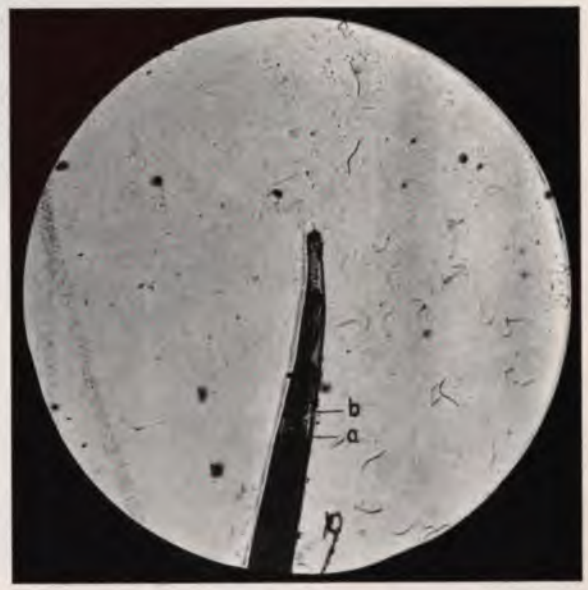

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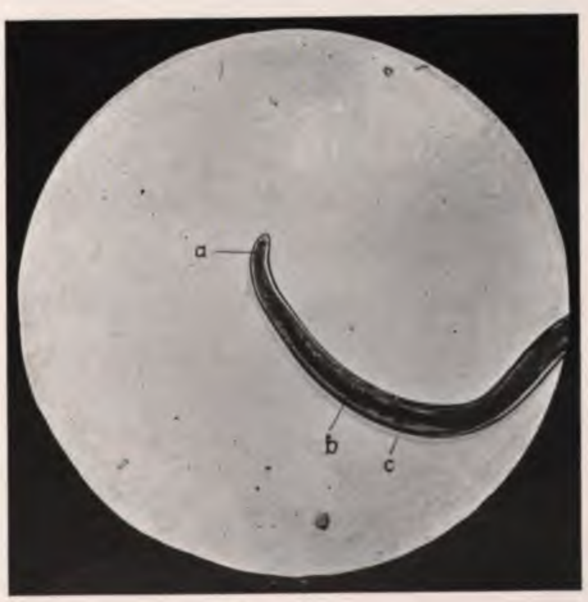

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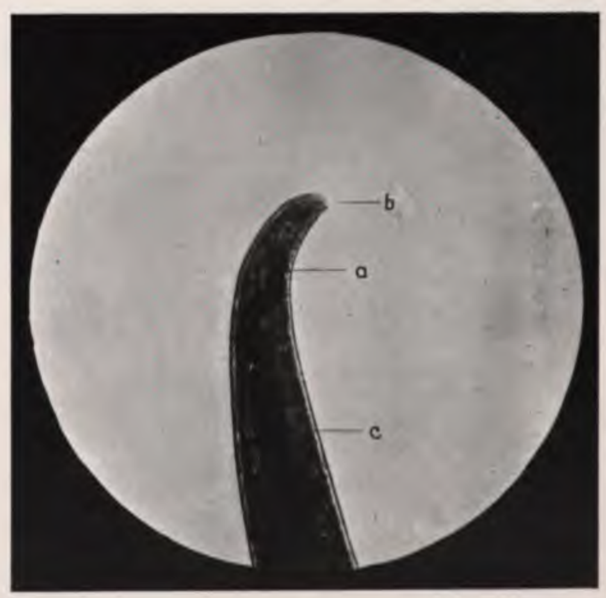

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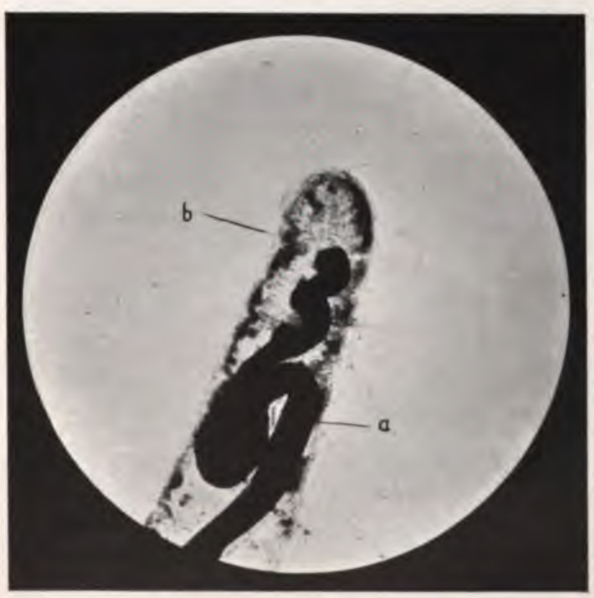

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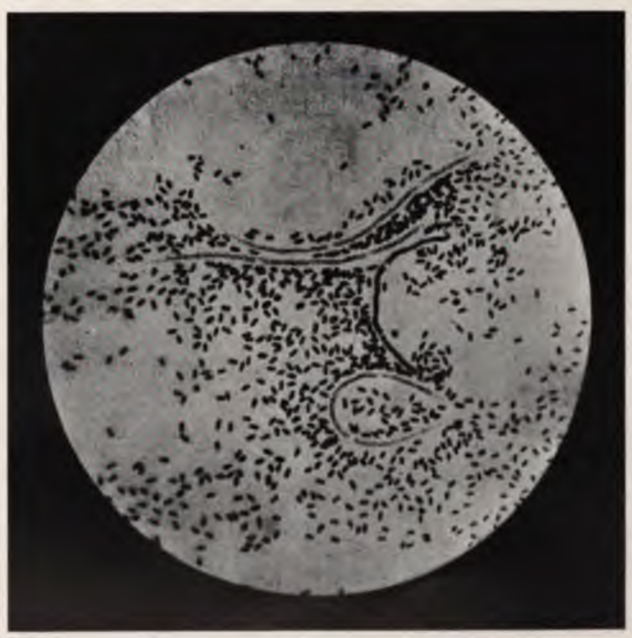

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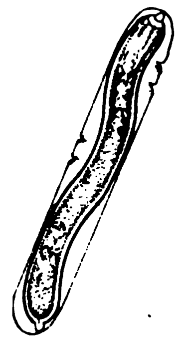

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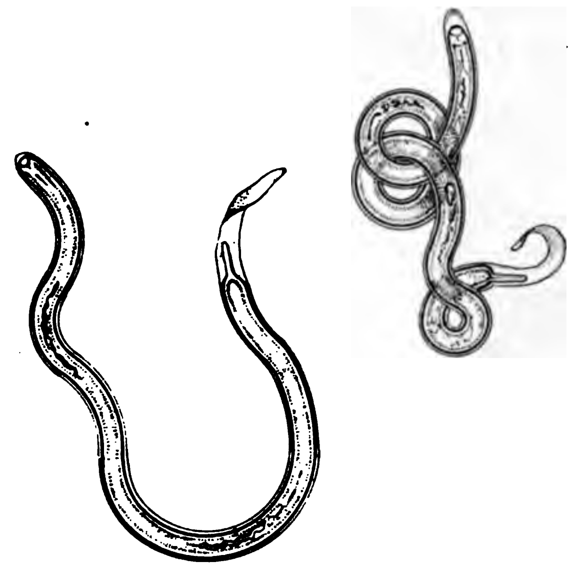

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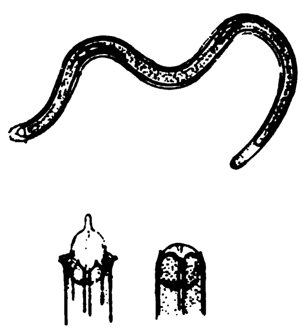


Fig. 3.

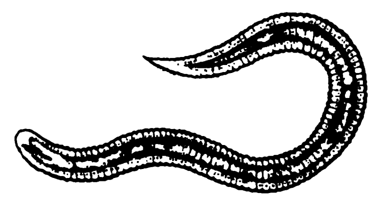
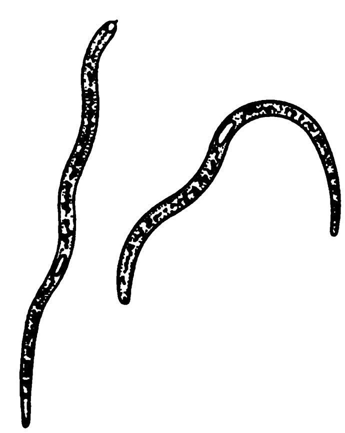


Fig. 4.



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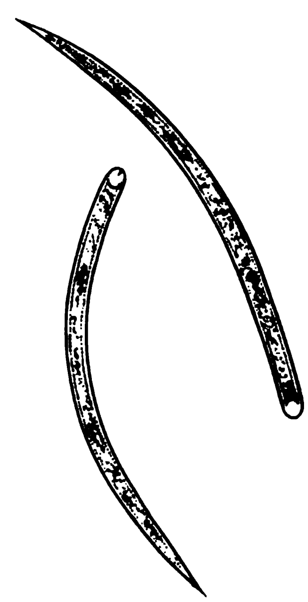

Fig 6.

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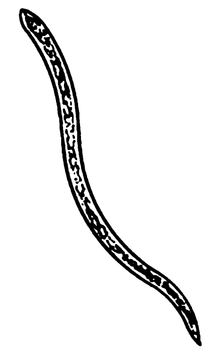

Fig. 7.

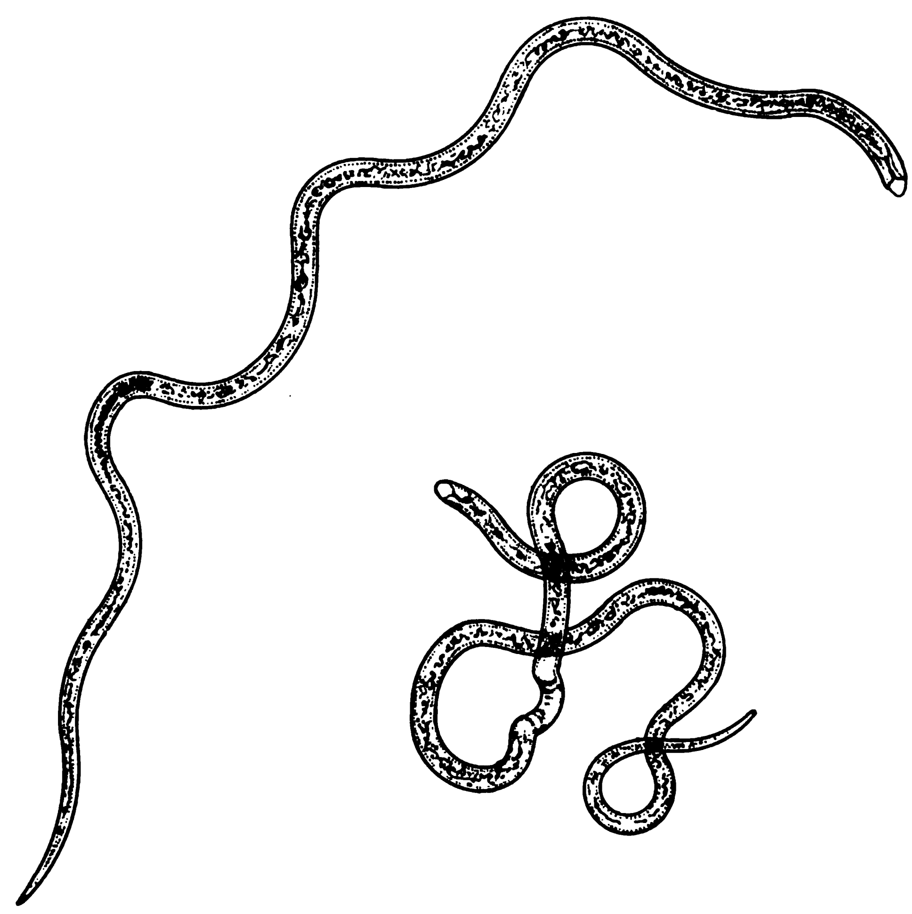

Fig. 8.

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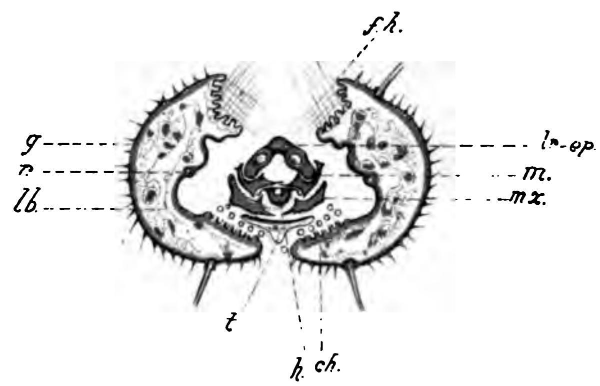


Fig 1.

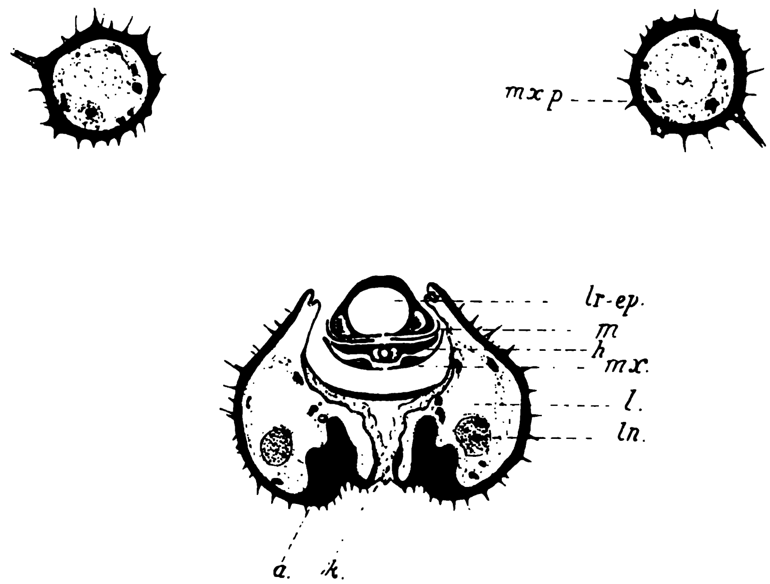
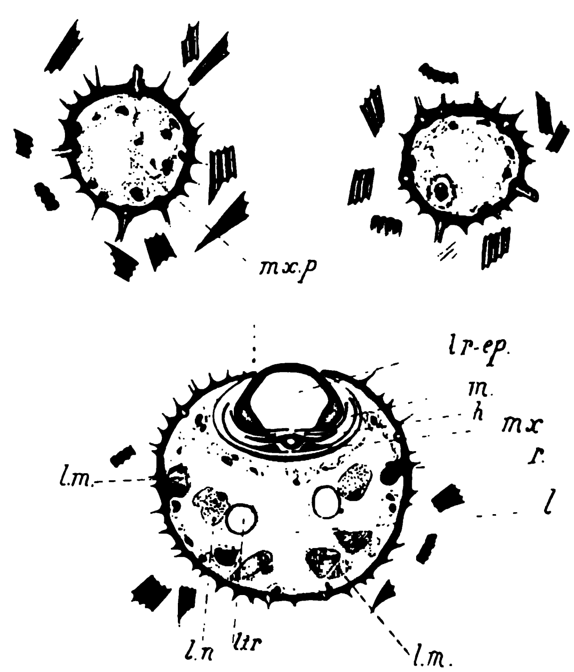


Fig 2.



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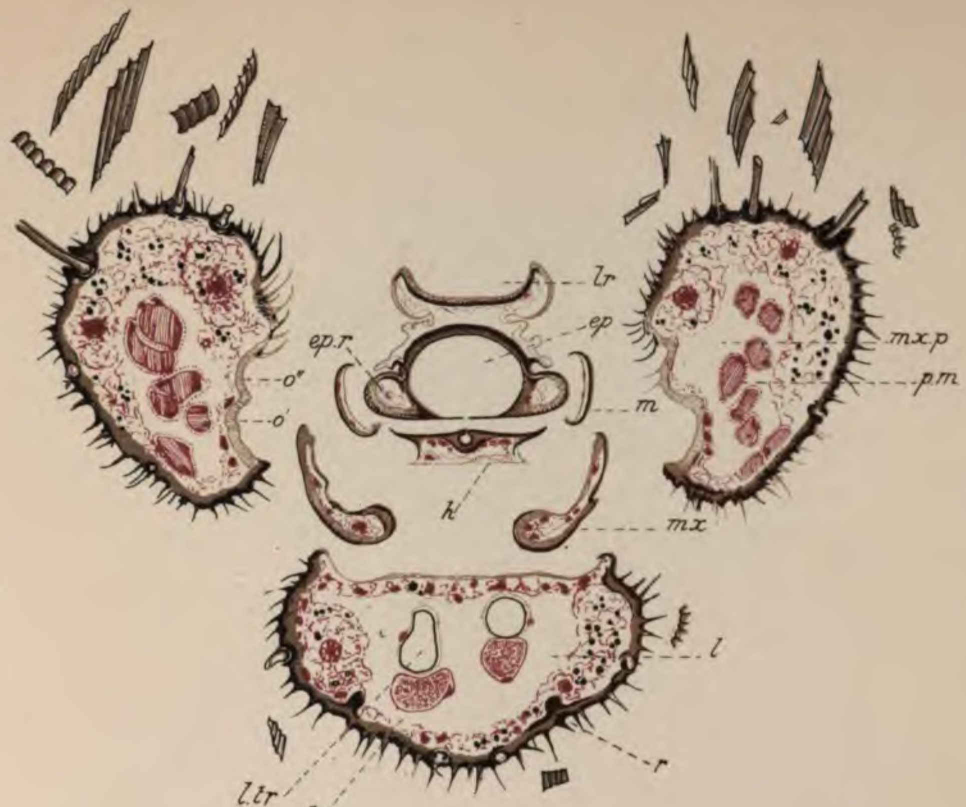

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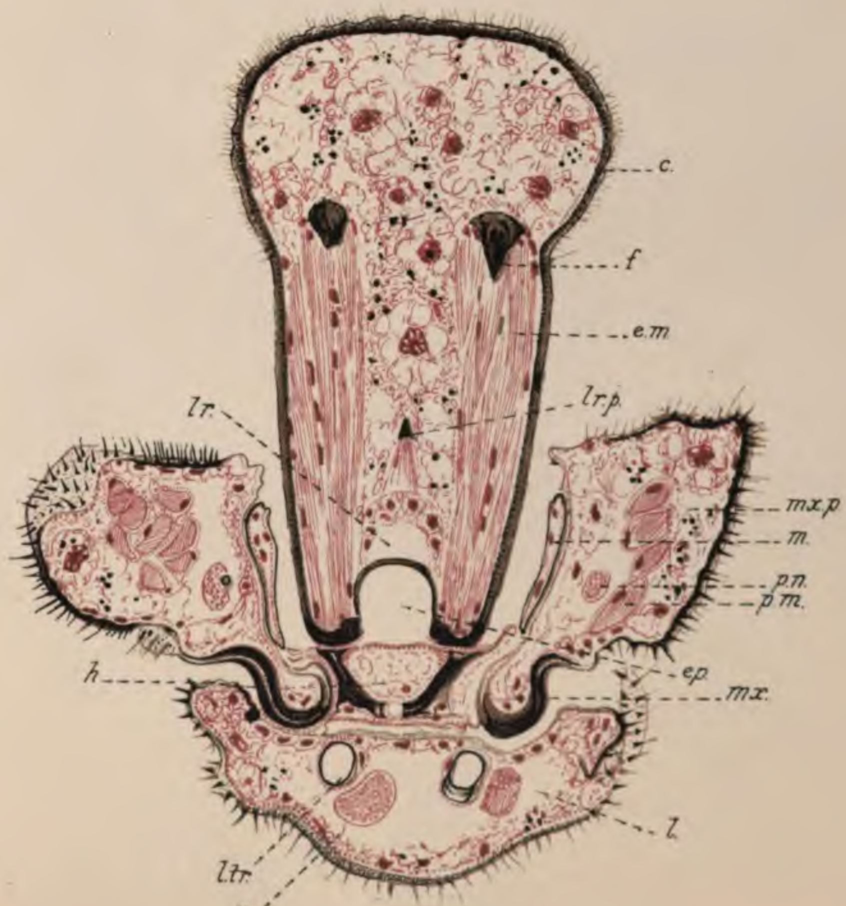

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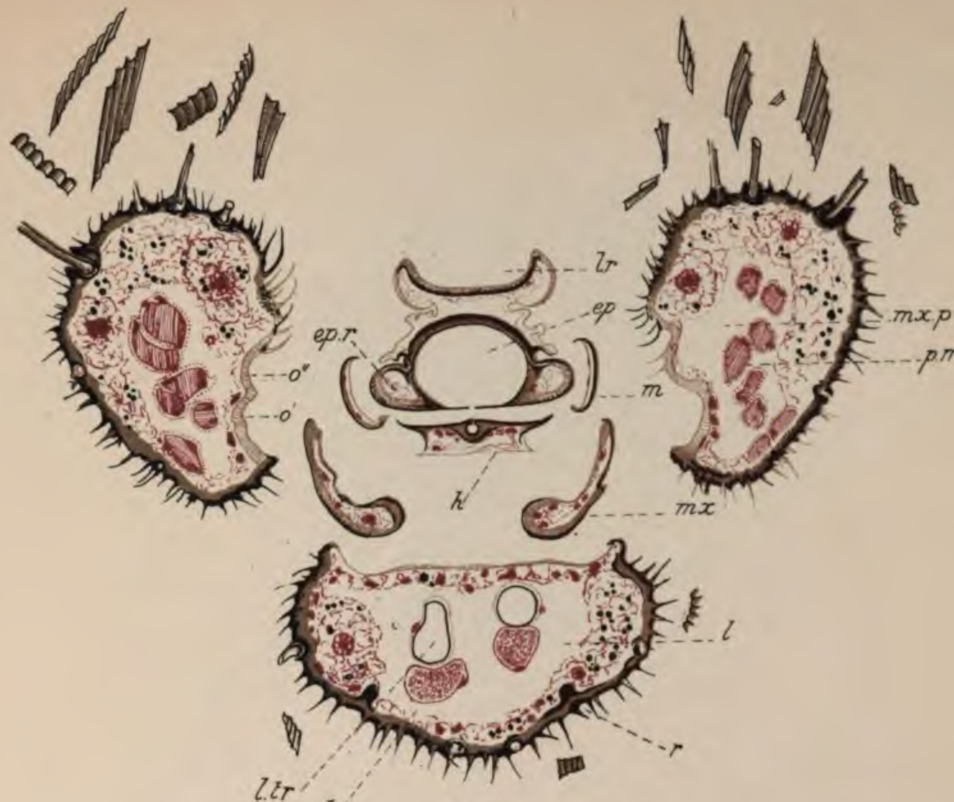
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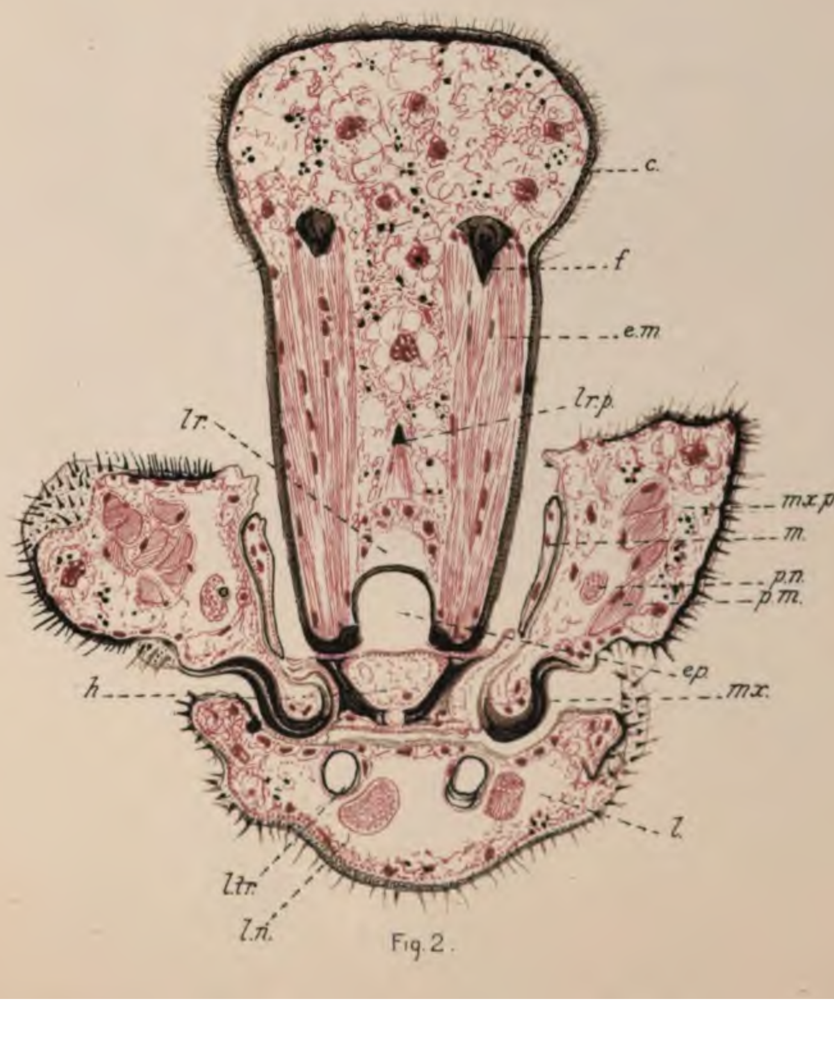
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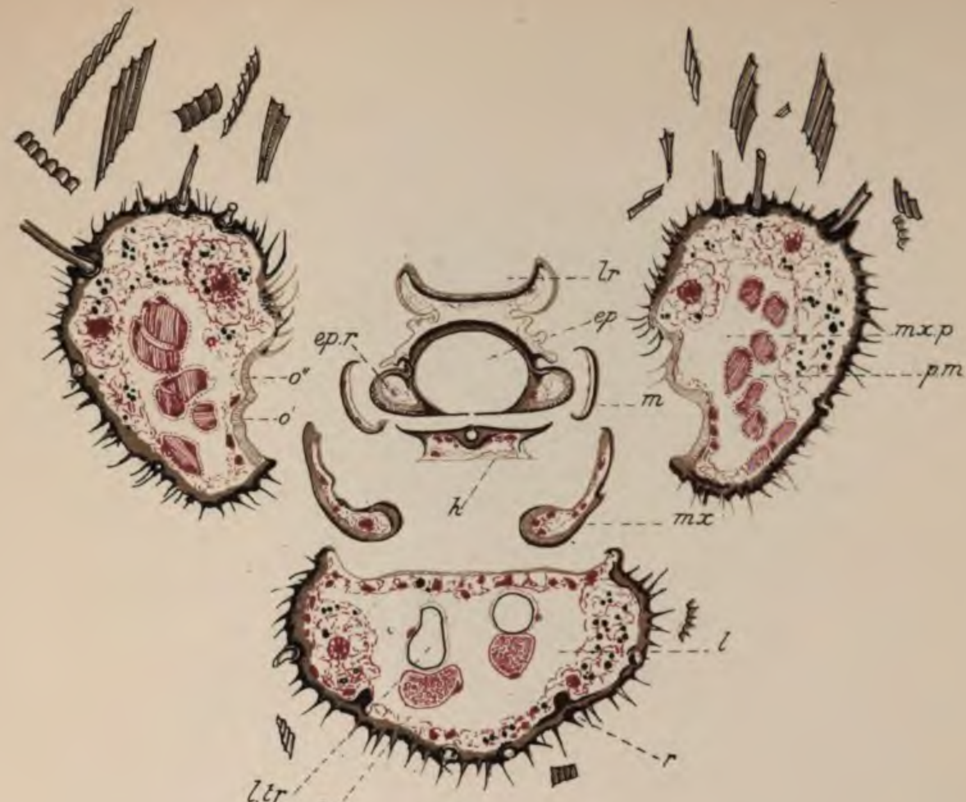
Fig. 2.



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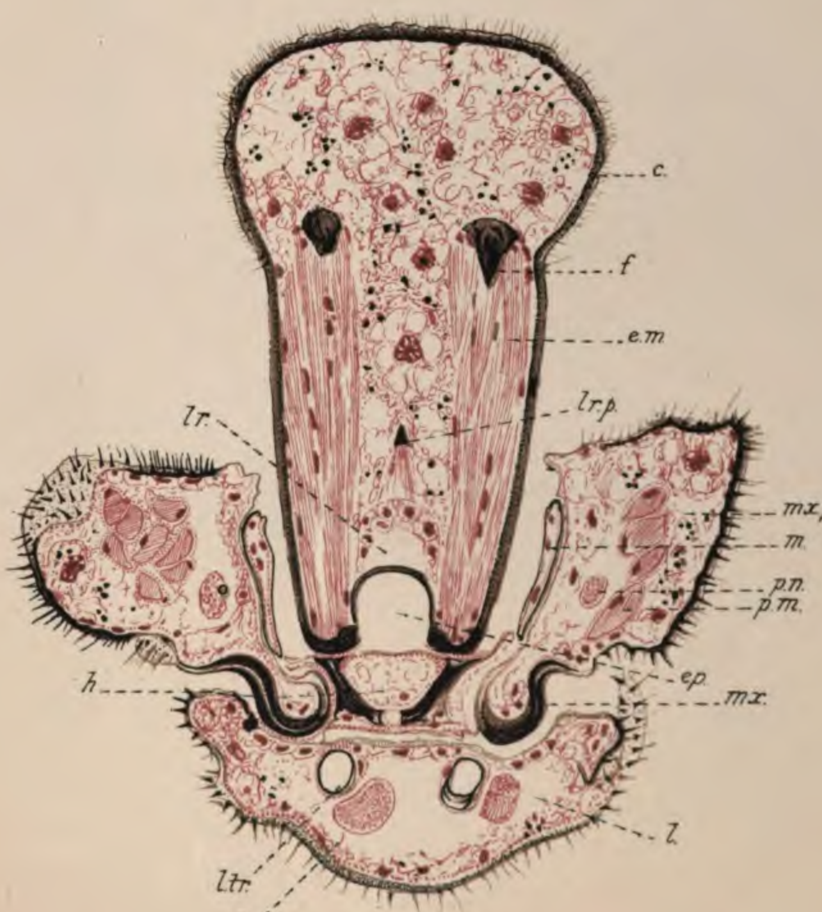

Fig. 2.

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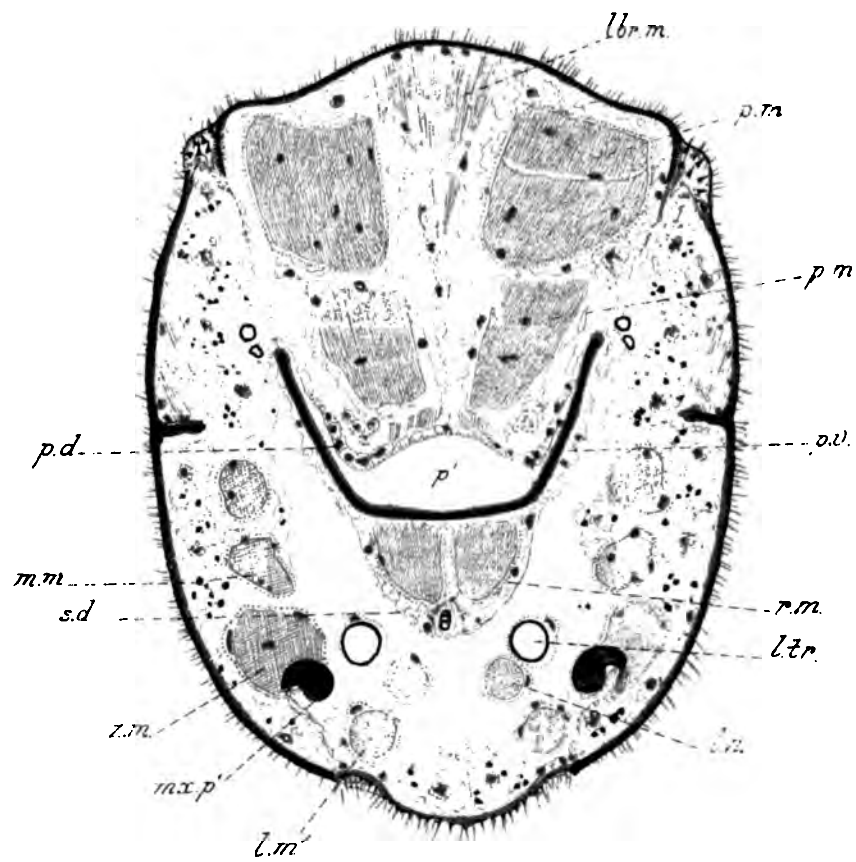

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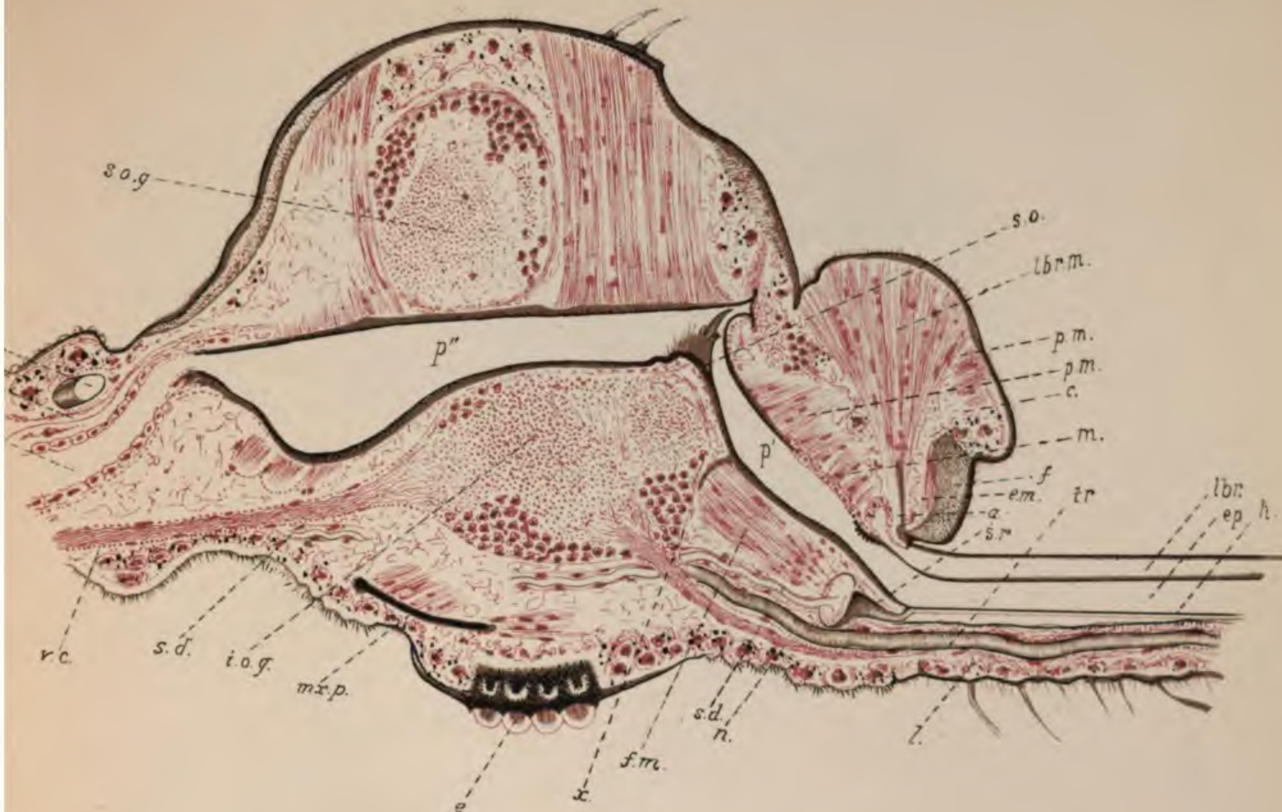

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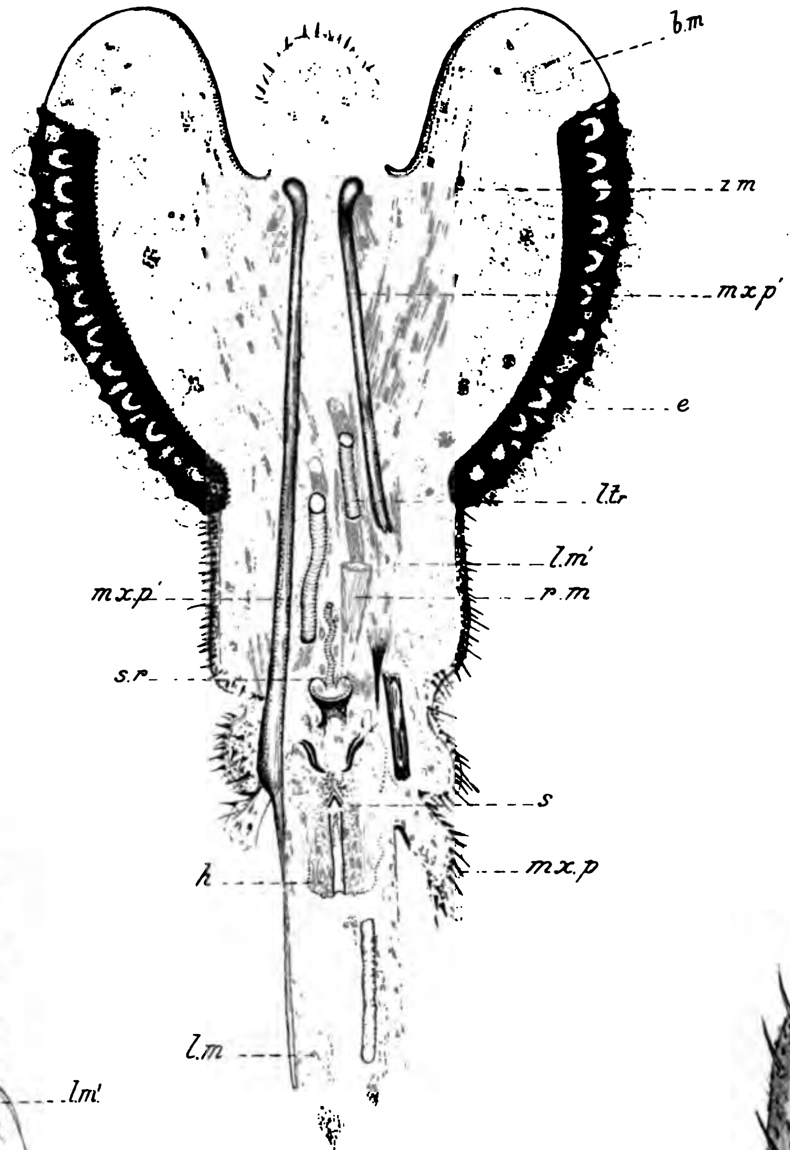
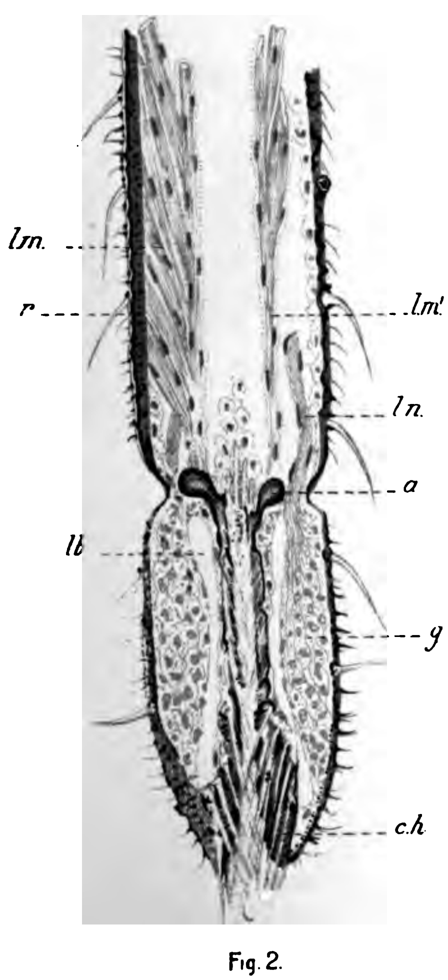


Fig 1



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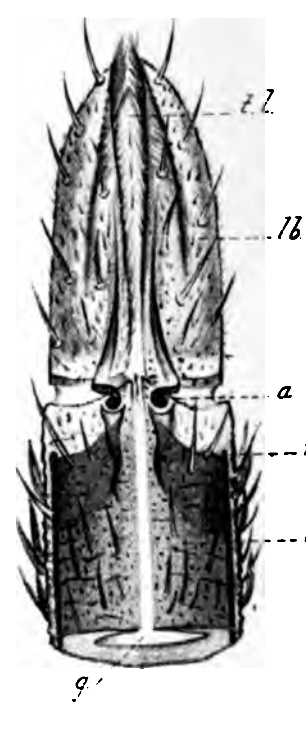

Fig 3

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BIBLIOGRAPHY

BIBLIOGRAPHY

COMPLETE LIST OF FILARIAE*

I. Larval Forms

Name	Literature	Host	Site
	(a) Mammalia		
F. diurna. Manson	See chapter iv	*Homo sapiens*	See chapter iv
F. perstans. Manson	” ”	*Homo sapiens*	” ”
F. vesperuginis. Linstow	Linstow. Arch. f. Naturg, li, 1885, p. 243	*Vesperugo serotinus* (Hameln)	In long oval cysts in the intestinal wall
F. irritans. Rivolta	Railliet. Zool. medic. et agric., Paris, 1893, p. 508	*Equus caballus. Equus asinus*	See chapter ii
	(b) Aves		
F. gruis. Linstow	Linstow. Arch. f. Naturg., xli, 1875, p. 197	*Ciconia alba*; *Grus cinerea*	Encysted either in stomach or intestinal wall
F. strigis. Linstow	Linstow. Arch. f. Naturg., xliii, 1877, p. 176; xlv, 1879, p. 173; xlvi, 1880, p. 45; xlviii, 1882, p. 1; li, 1885, p. 244	*Buteo vulgaris* (Hameln) *B. lagopus* (Hameln) *Otus vulgaris* (Hameln) *Nisus communis* (Hameln) *Astur palumbarius* (Hameln) *Bubo maximus* (Hameln) *Surnia noctua* (Hameln) *Strix flammea* (Hameln) *Surnia ulula* (Hameln) *Lanius excubitor* (Hameln)	
	(c) Pisces		
F. bicolor. Linstow	Linstow. Arch. f. Naturg., xxxix, 1873, p. 298	*Silurius glanis* (Hameln)	Under the peritoneal layer of stomach
	(d) Arthropoda		
F. stomoxeos. Linstow	Linstow. Arch. f. Naturg., xli, 1875, p. 195	*Stomoxys calcitrans* (Hameln)	In the proboscis
F. ephemeridarum. Linstow	Linstow. Arch. f. Mikr. Anat. xxxix, 1892, p. 396	*Ephemera vulgata* (Göttingen) *Oligoneura rhenana* (Göttingen)	
F. geotrupis. Linstow	Linstow. Arch. f. Mikr. Anat., xlviii, 1896, p. 375	*Geotrupis sylvaticus* (Göttingen)	
F. glomeridis. Linstow	Linstow. Arch. f. Naturg., li, 1885, p. 243	*Glomeris limbata* (Hameln)	
F. pulicis. Linstow	Linstow. Jena. Zeitsch, xxviii, 1893, p. 340	*Gammarus pulex* (Göttingen)	
F. gammari. Linstow	Linstow. Arch. f. Mikr. Anat., xxxix, 1892, p. 325	*Gammarus pulex* (Göttingen)	

II. Oral Aperture without Lips

Name	Literature	Host	Site
	(a) Mammalia		
F. bancrofti. Cobbold	See chapter iv	*Homo sapiens*	See chapter iv
F. loa. Guyot	See chapter iv	*Homo sapiens*	See chapter iv
F. lentis. Diesing	Diesing. Syst. Helm., ii, 1851, p. 265 Molin. Wien. Sitzber, xxviii, 1858, p. 390 Diesing. Wien. Sitzber, xlii, 1860, p. 702 Cobbold. Entoz. London, 1864, p. 332 De Bonis. Paras. d. corpo umano, Napoli, 1876, p. 129 Davaine. Traité d. Entoz., Paris, 1877, p. 106 Küchenmeister et Zürn. D. Paras d. Mensch, Leipzig, 1881, p. 429 Railliet. Zool. med. et agric., Paris, 1893, p. 529	*Homo sapiens* (Berlin)	See chapter ii
F. labialis. Pane	Pane. Ann Accad. d. Aspiranti Natur., Naples, 1864, p. 32 Davaine. Traité d. Entoz., Paris, 1877, p. cvii Küchenmeister u. Zürn. Paras d. Mensch, Leipzig, 1881, p. 430 Railliet. Zool. med. et agric., Paris, 1893, p. 530 Parona. Elminth italiana, Genova, 1894, p. 239	*Homo sapiens* (Naples)	See chapter ii

* From Stossich, *Filarie e Spiroptere*. Trieste, 1897.

Name	Literature	Host	Site
F. medinensis. Gmelin	Gmelin. Syst. Natur., 1878, p. 3039	*Homo sapiens* (Arabia, Persia,	See chapter ii
	Rudolphi. Entoz. Synops., 1819, p. 3	Turkestan, India, Guinea,	
	Lamark. Anim. s. vert., iii, 1840, p. 667	Senegambia, Darfur, Sennaar,	
	Dujardin. Hist. Nat. d. Helm., 1845, p. 44	Abyssinia, Nubia, Egitto,	
	Diesing. Syst. Helm., ii, 1851, p, 269	Curacao, Brazil); *Bos*	
	Baird. Catal. of Entoz., London, 1853, p. 5	*taurus*, *Equus caballus*	
	Molin. Wien. Sitzber, xxviii, 1858, p. 403	(India); *Canis familiaris*	
	Schneider. Monogr. d. Nemat, 1866, p. 85	(Egitto, Buenos Ayres,	
	De Bonis. Paras. d. corpo umano, Naples, 1876, p. 128	Curacao, India); *Canis*	
	Davaine. Traité d. Entoz., Paris, 1877, p. cvii	*lupaster: C. aureus: Felis*	
	Küchenmeister u. Zürn. D. Paras. d. Mensch, Leipzig, 1881, p. 417	*catus*, *F. guttata* (Kordofan)	
	Zürn. Thier. Paras. uns. Haussaüget, Weimar, 1882, p. 249		
	Blanchard. Anim. Paras., introd. par l'eau, Paris, 1890, p. 71		
	Railliet. Zool. med. et agric., Paris, 1893, p. 500		
	Parona. Elminthol italiana, Genova, 1894, p. 239		
Syn. *F. aethopica*	Valenciennes. Compt. rend Acad. Sc., xliii, 1856, p. 259		
	Molin. Wien. Sitzber, xxviii, 1838, p. 373		
Dracunculus persarum	Diesing. Wien. Sitzber, xlii, 1860, p. 686		
	Diesing. Wien. Sitzber, xliii, 1861, p. 286		
	Huber. Bibliogr. I. klin. Helminth, München, 1894, p. 245		
Dracunculus aethiopicus	Diesing. Wien. Sitzber, xlii, 1860, p. 698		
Dracunculus medinensis	Cobbold. Entoz., London, 1864, p. 373		
	Sonsino. Pr. Verb. d. Soc. Toscana d. sc. nat., 12 mag., 1889		
F. lymphatica. Treutler	Railliet. Zool. med. et. agric., Paris, 1893, p. 530	*Homo sapiens* (Sicily)	See chapter ii
Syn. *F. hominis bronchialis*	Rudophi. Entoz. Synops., 1819, p. 7 and 215	*Equus caballus* (Milan)	
	Dujardin. Hist. Nat. d. Helm., 1845, p. 45	*Equus asinus*	
	Diesing. Syst. Helm. ii, 1851, p. 279		
	Molin. Wien. Sitzber. xxviii, 1858, p. 418		
F. apapillocephala	Condorelli. Roma, 1892		
F. conjunctivae	Addario. Ann. d. ottalmolog., xiv, 1885		
	Condorelli. Filar. apapill. Roma, 1892		
F. dubini	Condorelli. " " " "		
F. inermis	Grassi. Centr. f. Bakt. u Par. I, 1887, p. 617		
	Calundruccio. Anim. Paras. dell' uomo in Sicilia, 1889, p. 19		
	Railliet. Zool. medic. et agric. Paris, 1893, p. 528		
	Parona. Elmintol. italiana. Genova, 1894, p. 239		
F. oculi	Quadri. Cpt. rend. d. Congres. Ophthal. d. Bruzel., 1858, p. 153.		
F. oculi asini	Condorelli. Filar. apapill. Roma, 1892		
F. palpebralis	Pace. Giorn. d. sc. nat. ed. econ. Palermo, ii, 1866, p. 152		
	Condorelli. Filar. apapill. Roma, 1892		
F. palpebrarum	De Bonis. Paras. d. corpo. umano. Napoli, 1876, p. 130		
F. peritonaei hominis	Babesius. Arch. f. path. Anat. u. Physiol., 1880, p. 158		
	Condorelli. Filar. apapill. Roma, 1892		
Hamularia lymphatica	Treutler. Observ. Path. Anat., 1793, p. 10		
Tentacularia subcompressa	Zeder. Naturg. d. Eingw., 1803, p. 45		
F. lacrymalis. Gurlt	Gurlt. Pathol. Anat., i, 1831, p. 347	*Bos taurus*, *Equus caballus*	See chapter ii
	Gerber. Handb. d. allg. Anat., 1840, p. 211		
	Diesing. Syst. Helm. ii, 1851, p. 265		
	Molin. Wien. Sitzber. xxviii, 1858, p. 372		
	Diesing. Wien. Sitzber., xlii, 1860, p. 701		
	Moroni. Il. medico. veterinario. Torino, 1864, April		
	Davaine. Traité d. Entoz. Paris, 1877, p. 109		
	Zürn. Thier Paras. uns Haussäuget. Weimar., 1882, p. 248		
	Perroncito. I parass. dell'uomo e. d. anim. Milano, 1882, p. 32		
	Railliet. Zool. med. et agric. Paris, 1893, p. 527		
	Parona. Elmintol. italiana. Genova, 1894, p. 241		
F. recondita. Grassi	Grassi e. Calundruccio. Centr. f. Bakt. u. Paras. vii, 1890, p. 18	*Canis familiaris* (Catania)	See chapter ii
	Railliet. Zool. med. et agric. Paris, 1893, p. 573		
	Parona. Elmintol. italiana. Genova, 1894, p. 241		
F. palpebralis. Wilson	Railliet. Zool. med. et agric. Paris, 1893, p. 528	*Equus caballus*	See chapter ii
F. nasicola. Leuchart	Baird. Catal. of Entoz. London, 1853, p. 5	*Mustela foina*; *M. putorius*	In frontal and
Syn. *Spiroptera nasicola*	Dujardin. Hist. Nat. d. Helm, 1845, p. 88		ethmoid sinuses
	Diesing. Syst. Helm., ii, 1851, p. 225		
F. acutiuscula. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 388	*Dicotyles albirostris* (Brazil);	Between the viscera
	Stossich. Boll. Soc. Adriat. di Sc. Nat., xii, 1890, p. 56	*D. torquatus* (Brazil);	In the pectoral
	Railliet. Zool. med. et agr. Paris, 1893, p. 508	*Vulpus azarae*, *Canis*	muscles
	Parona. Elmintol. italiana. Genova, 1894, p. 241	*familiaris* (Venice)	

Name	Literature	Host	Site
F. hyalina. Linstow	Linstow. Arch. f. Naturg., 1890, p. 182	*Sorex vulgaris* (Göttingen)	Intestine
F. haemorrhagica. Railliet.	Railliet et Moussu. Compt. rend. d. l. Soc. d. Biolog., iv, 1892, p. 545	*Equus caballus*	See chapter ii
	Railliet. Zool. med. et agric. Paris, 1893, p. 505	*Equus asinus*	
F. immitis. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 55	*Canis familiaris* (Europe, U.S.A., Brazil, Australia, Borneo, China, Japan); *C. lupus* (Japan); *C. vulpes*, *C. brachyucus*	In right heart, pulmonary arteries, and sometimes in other veins and arteries; occasionally free in thoracic cavity, in liver and subcutaneous and inter-muscular tissue
	Molin. Wien. Sitzber, xxviii, 1858, p. 384		
	Schneider. Monogr. d. Nematod., 1866, p. 87		
	Railliet. Journ. d. Veter. d. Midi., 1862, p. 49		
	De Silvestri. Il medico veterinario, ser. iii, vol. vi, 1871, p. 343		
	Ercolani. Mem. R. Acad. d. Sc. Bologna, series iii, tom. v, 1874, p. 390		
	Lewis. Quart. Journ. of micr., s. xv, 1875, p. 268		
	Davaine. Traité d. Entoz. Paris, 1877, p. 108		
	Rivolta. Giorn. Anat. fisiol. patol. anim., dom. ix, 1879, p. 17		
	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1880, p. 10		
	Zürn. Thier. Paras. uns Haussäuget. Weimar, 1882, p. 243		
	Megnin. Journ. d. l'Anat., xix, 1883, p. 172		
	Blanchard. Bull. Soc. Zool. di France, xii, 1887		
	Parona. Elmintol. sarda. Genova, 1887, p. 86		
	Sonsino. Sugli ematozoi del. cane. Pisa, 1888		
	Railliet. Zool. med. et agric. Paris, 1893, p. 509		
	Parona. Elmintol. italiana. Genova, 1894, p. 240		
	Ward. The paras. worms of man and the dom. anim., 1894, p. 319		
	Parona. Boll. d. Musei d. R. Univ. d. Genova, 1896, n. 43		
	Galli-Valerio. Moderna Zooiatro, 1897		
Syn. *F. canis cordis*	Leidy. Proc. Acad. Nat. Sc. Philadelphia, v, 1853, p. 118		
F. papillicauda	Molin. Wien. Sitzber, xxviii, 1858, p. 380		
" "	Diesing. Wien. Sitzber, xlii, 1860, p. 701		
F. flexuosa. Wedl	Wedl. Wien. Sitzber, xix, 1852, p. 122	*Cervus elaphus* (Vienna)	In subcutaneous tissues
	Molin. Wien. Sitzber, xxviii, 1858, p. 386		
	Linstow. Würtemb. naturw. Jahreshefte, 1879, p. 328		
F. crassicauda. Creplin	Creplin. Nov. Act. Nat. Cur., xiv, 1829, p. 874	*Balaenoptera rostrata* (Rugen) *Balaena mysticetus*	In the corpus cavernosus of the penis
	Dujardin. Hist. Nat. d. Helm, 1845, p. 50		
	Diesing. Syst. Helm., ii, 1851, p. 264		
	Molin. Wien. Sitzber, xxviii, 1858, p. 374		
	Beneden. Bull. Acad. Roy. Bruxelles, ser. ii, tom. xxix, 1870, p. 356		
F. quadrispina, Diesing	Diesing. Syst. Helm., ii. 1851, p. 271	*Mustela martes* (Pavia) *M. foina* (Trieste, Cittanova in Istria, Padua, Genova), *M. putorius* (Padua), *Hystrix cristata* (Senaar), *Gulo barbatus* (Brazil), *Galictis barbara*	Under the skin, in the pericardial sac; in the cavity of the abdomen
	Schneider. Monog. d. Nemat, 1866, p. 85		
	Stossich. Boll. Soc. Adriat. di Sc. Nat. Trieste, vii, 1890, p. 56 ; xiv, 1893, p. 85 ; xvii, 1896, p. 122		
Syn. *F. perforans*	Molin. Wien. Sitzber, xxviii, 1858, p. 387		
	Molin. " " xxx, 1858, p. 155		
	Diesing. " " xlii, 1860, p. 700		
	Molin. Denkschr. Wien. Akad., xix, 1861, p. 316		
	Diesing. Wien. Sitzber, xliii, 1861, p. 280		
	Parona. Ann. Museo. civico di Genova, 1887, p. 495		
	Parona. Elmintol. italiana. Genova, 1894, p. 240		
	Parona. Boll. d. Musei d. Univ. Torino, xi, 1896, n. 258		
F. martis	Gmelin. Syst. Nat. Lipsiae, 1788, p. 3040		
	Zeder. Naturg. d. Eingw., 1803, p. 38		
F. mustelarum subcutanea	Rudolphi. Entoz. Synops., 1819, p. 7 and 216		
F. australis. Linstow	Linstow. Arch. f. Mikr. Anat., xlix, 1897, p. 610	*Petrogale penicillata* (Australia)	Visceral cavity

(*b*) Aves

F. calamiformis. Schneider	Schneider. Monogr. d. Nemat., 1866, p. 90	*Psitacus aestivus* (Brazil)	Above the tendons of the feet
F. mazzantii. Railliet.	Railliet. Zool. med. et agric. Paris, 1893, p. 532	*Columba domestica*	Under the skin of the neck
F. schneideri. Stossich			
F. obtusoraudata	Schneider. Monogr. d. Nemat., 1866, p. 101	*Falco subbuteo* (Berlin)	Stomach
F. urogalli. Linstow	Linstow. Württemb. Naturw. Jahresh, 1879, p. 325	*Tetrao urogallus*	Under the skin
F. mansoni. Cobbold	Magalhäes. Revista Brazil d. Medicina, i, 1888, p. 5	*Gallus domesticus* (China, Rio Janeiro)	Orbital cavity
	Railliet. Zool. med. et agric., Paris, 1893, p. 533		
	Magalhäes. Bull Soc. Zool. de France, xx, 1895, p. 241		

Name	Literature	Host	Site
F. foveolata. Molin	Molin. Wien. Sitzber, xxviii, 1852, p. 375 Linstow. Arch. f. Naturg., xlv., 1879, p. 172 Stossich. Hist. Natur., Croat, vi, 1891, p. 217 Stossich. Hist. Natur., Croat, vii, 1892, p. 72	*Circus cyaneus*; *Falco peregrinus* (Trieste, Venice, Hameln); *F. lunarius*; *F. lithofalco*; *Nisus communis* (Venice); *Corvus frugilegus* (Switzerland); *Thamnophilus stagurus* (Brazil)	In pleural and abdominal cavities
F. nodulosa. Rudolphi	Diesing. Syst. Helm., ii, 1851, p. 274 Molin. Wien. Sitzber, xxviii, 1858, p. 409 Schneider. Monogr. d. Nemat., 1866, p. 91 Linstow. Arch. f. Naturg., xlix, 1883, p. 287 „ Vermi. Mosca, 1886, p. 12 Leidy. Proc. Acad. Nat. d. Sc. Philadelphia, 1886, p. 309 Parona. Elmintol. sarda Genova, 1887, p. 87 „ Ann. Museo civico di Genova, 1887, p. 495 Stossich. Boll. Soc. Adriat. di sc. Nat. Trieste, xii, 1890 „ Soc. Hist. Nat. Croat., vii, 1892, p. 72 „ Boll. Soc. Adriat. di Sc. Nat. Trieste, xiv, 1893 Parona. Elmintol. italiana. Genova, 1894, p. 242 Stossich. Boll. Soc. Adriat. di sc. Nat. Trieste, xvii, 1896, p. 122	*Lanius collurio* (Trieste, Venice, Berlin, Turkestan); *L. rufus* (Cagliari, Genova); *L. auriculatus* (Cagliari, Genova); *L. minor*; *Collurio lodovicianus* (Florida)	Under the skin of the cranium and back, and in oesophageal wall
Syn. *Tentacularia cylindrica*	Zeder. Naturg. d. Eingw., 1803, p. 45		
F. collurionis pulmonalis	Rudolphi. Entoz. Synops., 1819, pp. 8 and 217		
„ *subcutanea*	„ „ „ 1819, pp. 8 and 217		
F. obtusocaudata. Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 634 Dujardin. Hist. Nat. d. Helm., 1845, p. 55 Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien Sitzber, xxviii, 1858, 413 Linstow. Württemb. naturw. Jahresh., 1879, p. 327 „ Arch. f. Naturg., xlix, 1883, p. 284 „ Vermi. Mosca, 1886, p. 10 Parona. Ann. Museo civico di Genova, xxvii, 1889, p. 762	*Lanius rufus*; *L. minor*; *Pernix leucostriata*; *Picus flavescens*; *P. robustus*; *P. lineatus*; *P. passerinus*; *P. aurulentus*; *P. leucolaemus*; *P. iumana*	In the muscles of the neck, and in the thoracic cavity
Syn. *Monopetalonema obtuse-caudatum*	Diesing. Wien. Sitzber, xlii, 1860, p. 710		
F. spermospizae. Linstow.	Linstow. Arch. f. Naturg., xlv, 1879, p. 171	*Spermospiza guttata*	Internal cavities
F. bhamoensis. Parona	Parona. Ann. Museo civico di Genova, 1890, p. 777	*Acridotheres albocinctus* (Birmania)	In abdominal cavity
F. paronai. Stossich			
Syn. *F. sp.*	Parona. Ann. Museo civico di Genova, 1885, p. 433	*Buceros nasutus* (Soudan)	Kidney
F. sp.	Linstow. Arch. f. Naturg., 1891, p. 300		
F. ecaudata. Örley	Örley. Ann. Mag. of Nat. Hist., 1882, p. 312 Linstow. Arch. f. Naturg., 1891, p. 300	*Lamprotornis aeneus*	
F. clava. Wedl	Wedl. Wien. Sitzber, xix, 1856, p. 126 Molin. Wien. Sitzber, xxviii, 1858, p. 374 Diesing. Wien. Sitzber, xlii, 1860, p. 701 Ralliet. Zool. med. et agric. Paris, 1893, p. 532	*Columba domestica*	See chapter ii
F. tricuspis. Fedtschenko	Linstow. Arch. f. Naturg., xlix, 1883, p. 285 Linstow. Vermi. Mosca, 1886, p. 10 Linstow. Arch. f. Naturg., 1891, p. 293 Stossich. Boll. Soc. Adriat. di sc. nat. Trieste, xvii, 1896, p. 122	*Corvus cornix* (Venice, Padua, Vienna, Turkestan); *C. corone* (Vienna), *C. frugilegus* (Padua, Trieste, Vienna); *C. corax* (Vienna); *C. monedula* (Vienna); *Pyrrhocorax alpinus* (Vienna); *Pica caudata* (Vienna); *Garrulus glandarius* (Vienna); *Nucifraga caryocatactes* (Vienna); *Sturnella ludoviciana*; *Sylvia atricapilla* (Zagabria); *Poecile palustris* (Zagabria); *Corvus cyanomelas*; *Hirundo rustica*; *Alauda arvensis* (Trieste); *Lullula arborea* (Trieste); *Acridotheres tristis* (East Indies); *A. ginginianus* (East Indies)	Abdominal cavity
Syn. *F. unguiculata*	Rudolphi. Entoz. Synops., 1819, pp. 4 and 209 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 267 Molin. Wien. Sitzber, xxviii, 1858, p. 378		
F. monticelliana	Stossich. Soc. Hist. Nat. Croat., v, 1890, p. 130		
F. ninnii	Stossich. Soc. Hist. Nat. Croat., vi, 1891, p. 217		
F. alaudae	Zeder. Natur. d. Eingw., 1803, p. 39		
F. flabellata. Linstow	Linstow. Zool. of the voyage of H.M.S. Challenger, vol. xxiii, part lxxi, London, 1888, p. 9 Linstow. Arch. f. Naturg., 1891, 300 Parona. Ann. Museo civico di Genova, 1890, p. 777	*Paradisea apoda* (Aru Island); *Cyanops ramsayi* (Tenasserim)	Subcutaneous, and in internal cavities
F. pungens. Schneider	Schneider. Monogr. d. Nemat., 1866, p. 92 Linstow. Arch. f. Naturg., 1891, p. 300	*Turdus cyaneus* (Argo)	
F. obtusa. Rudolphi	Zeder. Naturg. d. Eingw., 1803, p. 36 Rudolphi. Entoz. Synops., 1819, p. 4 Dujardin. Hist. nat. d. Helm., 1845, p. 53 Diesing. Syst. Helm., ii, 1851, p. 267 Baird. Catal. of Entoz. London, 1853, p. 6 Molin. Wien. Sitzber, xxviii, 1858, p. 397 Diesing. „ „ xlii, 1860, p. 702	*Hirundo rustica* (Vienna, Greifswald, Rennes); *H. urbica* (Genova, Vienna, Turkestan); *H. riparia* (Vienna); *H. versicolor* (Brazil); *Progne purpurea*; *Myothera caudacuta*	Abdominal cavity

Name	Literature	Host	Site
F. obtusa. Rudolphi—contd.	Linstow. Arch. f. Naturg., xlix, 1883, p. 286		
	Linstow. Vermi. Mosca, 1886, p. 11		
	Parona. Ann. Museo civico di Genova, 1887, p. 495		
	Linstow. Arch. f. Naturg., 1891, p. 300		
	Parona. Elmintol italiana. Genova, 1894, p. 242		
F. turdi atrogalaris. Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 288	*Turdus atrogularis* (Turkestan)	
	Linstow. Vermi. Mosca, 1886, p. 13		

(*c*) Reptilia

Name	Literature	Host	Site
F. macrophallos. Parona	Parona. Ann. Museo civico d. Genova, 1890, p. 778	*Hydrosaurus salvator* (Birmania)	Between the abdominal muscles
F. emmae. Stossich Syn. *F. sp.*	Parona. Ann. Museo civico di Genova, 1890, p. 778	*Calotes emma* (Burmah)	
F. dahomensis. Neumann	Neumann. Bull. Soc. Zool. de France, xx, 1895, p. 123	*Python natalensis* (Dahomey)	In the subcutaneous tissue of the abdominal wall

(*d*) Pisces

Name	Literature	Host	Site
F. denticulata. Rudolphi	Schneider. Monog. d. Nemat., 1866, p. 102	*Anguilla vulgaris*	Stomach
Syn. *Cochlus inermis*	Zeder. Naturg. d. Eingw., 1803, p. 50		
Liorhynchus denticulatus	Rudolphi. Entoz. Synops, 1879, pp. 62 and 307		
	Bremser. Icon. Helminth, 1824		
	Lamark. Anim. s. vert., iii, 1840, p. 646		
	Dujardin. Hist. Nat. d. Helm., 1845, p. 284		
	Diesing. Syst. Helm. ii, 1851, p. 247		
F. echinata. Linstow	Linstow. Arch. f. Naturg., xliv, 1878, p. 235	*Alburnis lucidus* (Hameln)	Intestine
F. obturans. Prenant	Prenant. Bull. Soc. sc. Nancy (2), vii, 1888, p. 215	*Esox lucius* (Nancy)	Bronchial artery

III. Buccal cavity has no lips, but is surrounded by a chitinous ring

(*a*) Mammalia

Name	Literature	Host	Site
F. equina. Abildgaard	Blanchard. Ann. d. Sc. Nat., ser. iii, tom. xi, p. 154	*Equus caballus* (Italy); *E. asinus* (Milan); *E. mulus*; *Bos taurus*; *B. bubalus*	In peritoneal cavity, testicle, pleural cavity, lungs, between the meninges, in liver, aqueous humour
	Railliet. Zool. med. et agric. Paris, 1893, p. 524		
	Neumann. Revue vétérin, xxii, 1897, p. 75		
Syn. *F. equi*	Gmelin. Syst. Natur., 1789, p. 3039		
F. papillosa	Zeder. Naturg. d. Eingw., 1803, p. 37		
	Anderson. Edinb. Medic. and Surg. Journal, ii, 1805, p. 306		
	Sick. Med. Jahrb. d. k. k. österr. Staat, ii, 1813, p. 174		
	Greve. Krankh. d. Hausth., 1818, p. 174		
	Rudolphi. Entoz. Synops., 1819, pp. 6 and 213		
	Bremser. Vers intest. d. l'hom, 1824, p. 123		
	Bremser. Icon. Helminth, 1824		
	Gurlt. Path. Anat. i, 1831, p. 348		
	Nordmann. Microgr. Beiträge, 1832, p. 11		
	Siebold. Arch. f. Naturg., 1837, p. 255		
	Lamark. Anim. s. vert., iii, 1840, p. 668		
	Dujardin. Hist. Nat. d. Helm., 1845, p. 49		
	Diesing. Syst. Helm., ii, 1851, p. 272		
	Baird. Catal. of Entoz. London, 1853, p. 5		
	Wedl. Wien. Sitzber, xvii, 1855, p. 307; xix, 1856, p. 56		
	Leidy. Proc. Acad. Nat. Sc., Philadel., viii, 1856, p. 55		
	Molin. Wien. Sitzber, xxviii, 1858, p. 405		
	Schneider. Monogr. d. Nematod., 1866, p. 86		
	Panizza. Il medico veterinario. Torino, 1869, p. 193		
	Davaine. Traité d. Entoz. Paris, 1877, p. cix		
	Zürn. Their. Paras. uns. Haussäuget. Weimar, 1882, p. 242		
	Lange. Deutsch. Zeitschr. f. Thier medicin, viii, 1882, p. 71		
	Linstow. Arch. f. Naturg., xlix, 1883, p. 284		
	Linstow. Vermi. Mosca, 1886, p. 9		
	De Silvestri. Giorn. d. medic. veterin. prat. Torino, xxxvi, 1887, p. 429		
	Blanchard. Bull. Soc. Zool. France, xii, 1887		
	Parona. Ann. Museo civico di Genova, 1887, p. 494		
	Deupser. Zool. Anzeiger, 1892, n. 388		
	Parona. Elmintol. italiana. Genova, 1894, p. 241		
F. bubali	Rudolphi. Entoz. Synops., 1819, p. 8		
	Molin. Wien. Sitzber, xxviii, 1858, p. 421		
F. strumosa. Rudolphi	Schneider. Monog. d. Nematod., 1866, p. 103	*Talpa europaea* (Padua, Sondrio, Rennes, Vienna, Halle, Hameln, Greifswald, Ireland) (Larval form encysted in fat bodies of *Cetonia aurata*)	Stomach and intestines, and in pedunculated tumours on external coat of stomach
	Linstow. Arch. f. Naturg., 1885, p. 241		
	Linstow. Zool. Jahrb., iii, 1887, p. 112		
Syn. *Spiroptera strumosa*	Rudolphi. Entoz. Synops, 1819, pp. 24 and 241		
	Dujardin. Hist. Nat. d. Helm., 1845, p. 86		
	Diesing. Syst. Helm., ii, 1851, p. 213		
	Baird. Catal. of Entoz. London, 1853, p. 2		

[illegible]

[illegible]	[illegible]		
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]		

[illegible]

[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	Under the skin of the neck
[illegible]	[illegible]	[illegible]	Thoracic and abdominal cavities
[illegible]	[illegible]		
[illegible]	[illegible]		
[illegible]	[illegible]		

IV. Buccal orifice with two lips

(a) Mammalia

[illegible] Muller	Muller [illegible]	*Felis domestica* (Bavaria)	In neighbourhood of end of oesophagus and cardia of stomach
[illegible]	[illegible]	*Paradoxurus philippinensis*	Stomach

(b) Aves

[illegible]	[illegible]	*Rhea americana* (Brazil)	Stomach; thoracic and abdominal cavities, muscles, under the skin, and in ovum
[illegible]	[illegible]		

Name	Literature	Host	Site
F. paradiseae. Linstow	Linstow. The Zool. of the voyage of H.M.S. Challenger, Entoz., 1888, p. 11	*Paradisea apoda* (Aru Island)	
F. tridentata. Linstow	Linstow. Arch. f. Naturg., xliii, 1877, pp. 10 and 175	*Colymbus articus* (Hameln) *Larus ridibundus*	Intestine Oesophagus
F. rotundata. Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 283 Linstow. Vermi. Mosca., 1866, p. 8	*Otis mac quini* (Turkestan)	
F. recta. Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 324	*Podiceps cristatus*	In stomach wall
F. coelebs. Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 326	*Lanius rufus*	In stomach wall
F. capitella. Schneider	Schneider. Monogr. d. Nematod., 1866, p. 96	*Coracius garrula*	In stomach wall

(*c*) Pisces

Name	Literature	Host	Site
F. conoura. Linstow	Linstow. Arch. f. Naturg., li, 1885, p. 242	*Anguilla vulgaris* (Hameln)	Intestine

V. Buccal orifice with three or six lips

(*a*) Mammalia

Name	Literature	Host	Site
F. ascaroides. Linstow	Linstow. Württemb. Naturw. Jahresh., 1879, p. 332	*Cercopitherus mona*	Bronchi
F. muris. Gmelin			
Syn. *Ascaris muris* *Fusaria muris* *Filaria obtusa*	Gmelin. Syst. Nat., p. 3032 Zeder. Naturg. d. Eingw., 1803, p. 106 Schneider. Monogr. d. Nemat., 1866, p. 97 Linstow. Arch. f. Naturg., xlix, 1883, p. 286 Kowalwski. Sitzber. Akad. Krakau, xxi, 1896, p. 257	*Mus musculus* (Vienna, Galicia, Breslau, Griefswald, Berlin, Rennes); *Mus decamanus* (Brazil)	Stomach
Spiroptera obtusa	Rudolphi. Entoz. Synops, 1819, pp. 27 and 249 Bremser. Vers. intest. de l'hom., 1824, p. 126 Bremser. Icon. Helminth, 1824 Lamark. Anim. s. Vert., iii, 1840, p. 661 Dujardin. Hist. Nat. d. Helm., 1845, p. 89 Diesing. Syst. Helm., ii, 1851, p. 214 Baird. Catal. of Entoz. London, 1853, p. 9 Molin. Wien. Sitzber, xxxviii, 1859, p. 934 Parona. Elmintol. italiana. Genova, 1894, p. 246		
F. verrucosa. Molin			
Syn. *Spiroptera verrucosa*	Molin. Wien. Sitzber, xxxviii, 1859, p. 964 Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 203	*Cervus dichotomus* (Brazil); *C. nambi*; *C. paludosus*	Between the tendons of the phalanges

(*b*) Aves

Name	Literature	Host	Site
F. obvelata. Creplin	Linstow. Arch. f. Naturg., xliii, 1877, p. 174 Parona. Elmintol. Sarda. Genova, 1887, p. 88 Braun. Arch. d. Fr. d. Naturg. i. M., 1891, p. 112 Stossich. Soc. Hist. Natur. Croat, vii, 1892, p. 72 Parona. Elmintol. italiana. Genova, 1894, p. 243	*Larus medius*; *L. canus* (Warnemünde); *L. fuscus*; *L. marinus*; *L. ridibundus* (Cagliari, Trieste); *L. argentatus*; *L. argentatoides*;	Oesophagus and proventriculus
Syn. *Spiroptera obvelata* *Histiocephalus spiralis* *Cosmocephalus alatus*	Dujardin. Hist. Nat. d. Helm., 1845, p. 101 Diesing. Spst. Helm., ii, 1851, p. 231 Molin. Wien. Sitzber, xl, 1860, p. 345 Diesing. Wien. Sitzber, xlii, 1860, p. 673	*L. maximus* (Griefswald); *Mergus serrator*; *Sterna risoria*; *Totanus fuscus* (Hameln); *T. maculatus*; *T. hypolencus*; *Alca torda*; *Ursa grylle*	
F. phasiani picti. Molin.			
Syn. *Spiroptera phasiani picti*	Molin. Wien. Sitzber, xxxviii, 1859, p. 981 Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 206	*Phasianus pictus* (Vienna)	Stomach wall
F. tulostoma. Hempr. et Ehr.	Schneider. Monogr. d. Nemat., 1866, p. 102	*Neophron percnopterus*	Thorax
F. vulturis. Molin			
Syn. *Spiroptera vulturis*	Molin. Wien. Sitzber, xxxviii, 1859, p. 976 Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 205.	*Cathartes papa* (Brazil)	Between the muscles of the lower jaw
F. anolabiata. Molin			
Syn. *Spiroptera anolabiata*	Molin. Wien. Sitzber, xxxviii, 1859, p. 981 Drasche. Zool. Botan. Gesell. Wien., xxxiii, 1884, p. 206	*Crax fasciolata* (Brazil)	Under the nictitating membrane
F. leptoptera. Rudolphi	Schneider. Monogr. d. Nemat., 1866, p. 97 Linstow. Arch. f. Naturg., xliii, 1877, p. 10 Linstow. Wurttemb. Naturw. Jahresh., 1879, p. 325 Kowalewski. Sitzber Akad. Krakaw, xxxi, 1896, p. 256	*Milvus regalis*; *M. ater* (Greifswald); *Accipites nisus* (Hameln, Ireland); *Circus cineraceus* (Vienna);	

NAME	LITERATURE	HOST	SITE
Syn. *Spiroptera leptoptera*	Rudolphi. Entoz. Synops., 1819, pp. 26 and 247 Dujardin. Hist. Nat. d. Helm., 1845, p. 93 Diesing. Syst. Helm., ii, 1851, p. 217 Baird. Catal. of Entoz. London, 1853, p. 10 Molin. Wien. Sitzber, xxxviii, 1859, p. 953	*C. cyaneus*; *C. rufus*; *Buteo vulgaris* (Vienna, Galicia, Berlin, Rennes); *Astur palumbarius*; *Herpetotheres cachinnans* (Brazil); *Harpagus bidentatus* (Brazil); *Falco tinnunculus* (Vienna); *F. lanarius*; *F. albicollis*; *F. subbuteo* (Vienna); *F. aurantius* (Brazil); *F. magnirostris* (Brazil); *F. xanthothorax* (Brazil); *F. unicinctus* (Brazil); *F. tridentatus* (Brazil);	Oesophagus, stomach and intestine
		Emberiza pecoris (Paris)	Under the palpebral conjunctiva
F. guttata. Schneider	Schneider. Monogr. d. Nemat., 1866, p. 92	*Falco berigora* (Adelaide)	
F. attenuata. Rudolphi	Rudolphi. Entoz. Synops., 1819 pp. 4 and 205 Bremser. Vers intest. d. l'hom., 1824, p. 123 Bremser. Icon. Helminth., 1824 Blanchard. Ann. d. Sc. Nat., ser. iii, tom., xi, p. 156 Lamark. Anim. s. Vert., iii, 1840, p. 667 Ecker. Arch. f. Anat. u. Phys., 1845, p. 503 Dujardin. Hist. Nat. d. Helm., 1845, p. 50 Diesing. Syst. Helm., ii, 1851, p. 266 Baird. Catal. of Entoz. London, 1853, p. 6 Wedl. Wien. Sitzber., xvii, 1855, p. 308, xix, 1856, p. 57 Leidy. Proc. Acad. Nat. Sc. Philadelph., viii, 1856, p. 56 Molin. Wien. Sitzber, xviii, 1858, p. 394; and xxx, 1858, p. 155 Diesing. Wien. Sitzber., xlii, 1860, p. 702 Molin. Denkschr. Wien. Akad., xix, 1861, p. 316 Diesing. Wien. Sitzber., xliii, 1861, p. 280 Schneider. Monogr. d. Nemat., 1866, p. 89 Stossich. Soc. Hist. Nat. Croat., vi, 1891, p. 217 Linstow. Arch. f. Naturg., 1891, p. 292	*Falco subbuteo*, *F. lanarius*, *F. peregrinus*, *Otus brachyotus*, *Strix torquata* (Brazil), *Garrulus glandarius* (Venice)	Thoracic and abdominal cavities and in the muscles
Syn. *F. falconis*	Gmelin. Syst. Nat., p. 3040 Zeder. Naturg. d. Eingw., 1803, p. 38		
F. nodispina	Molin. Wien. Sitzber., xxviii, 1858, p. 402 Diesing. Wien. Sitzber., xlii, 1860, p. 703		
F. quadripens	Molin. Wien. Sitzber., xxviii, 1858, p. 407 Diesing. Wien. Sitzber., xlii, 1860, p. 703		
F. strigis torquatae	Molin. Wien. Sitzber, xxviii, 1858, p. 422		

(c) Pisces

F. ochracea. Linstow.	Linstow. Jenaische Zeitsch, xxviii, 1893, p. 339	*Thymallas vulgaris* (Göttingen)	Stomach

VI. OTHER FORMS

(a) Mammalia

F. restiformis. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1880, p. 130 Railliet. Zool. méd. et agric. Paris, 1893, p. 530	*Homo sapiens*	See chapter ii
F. diacantha. Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 381	*Cercolabes prehensilis*, *Mesomys spinosus* (Brazil)	Abdominal cavity and lungs
F. felis mellivorae. Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 421	*Felis mellivora* (Brazil)	Lungs
F. felis onçae. Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 421	*Felis onça* (Brazil)	Between the muscles
F. filiformis. Molin	Molin. Zool. méd. et agric. Paris, 1893, p. 396 Diesing. Zool. méd. et agric. Paris, xlii, 1860, p. 702	*Anabates rufifrons* (Brazil)	Abdominal cavity
F. striata. Molin	Molin. Wien. Sitzber, 1858, p. 388	*Felis concolor*	Subcutaneous
Syn. *Solenonema striatum*	Diesing. Wien. Sitzber, xlii, 1860, p. 705	*F. macroura* (Brazil)	
F. scapiceps. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1886, p. 308	*Lepus sylvaticus* (N. America)	
F. serpicula. Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 385	*Corallia brevicaudum* *Phyllostoma spiculatum* (Brazil)	Abdominal cavity
Syn. *Solenonema serpiculum*	Diesing. Proc. Acad. Nat. Sc., Philadelphia, xlii, 1860, p. 705		
F. spirocauda. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1858, p. 112 Diesing. Wien. Sitzber, xlii, 1860, p. 701 Braun. Arch. d. F. d. Naturg., i, M. 1891, p. 112	*Phoca vitulina* (Pennsylvania, Warnemünde)	Heart
Syn. *F. cordis phocae*	Joly. Compt. rend. Acad. d. sc., xlvi, 1856, p. 403		
F. stigmatura. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, 1886, p. 309		

Name	Literature	Host	Site
F. conica. Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 412	*Dasyprocta aguti, Cavia acuschy* (Brazil)	Abdominal cavity
Syn. *Dicheilonema conicum*	Diesing. Proc. Acad. Nat. Sc., Philadelphia, xlii, 1860, p. 708		
F. canis brachyuri. Molin	Molin. Proc. Acad. Nat. Sc., Philadelphia, xxviii, 1858, p. 420	*Canis brachyuris* (Brazil)	Under the tracheal epithelium
F. caprae. Linstow	Linstow. Arch. f. Naturg., xlix, 1883, p. 287 Linstow. Vermi. Mosca., 1886, p. 12 Railliet. Zool. medic. et agric. Paris, 1893, p. 531	*Capra hircus* (Turkestan)	Muscles under the tongue
F. laevis. Creplin	Creplin. System. Helm., ii, 1851, p. 265 Molin. Wien. Sitzber, xxviii, 1858, p. 389 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Tarsius spectrum*	Under the skin
F. leonis. Gmelin	Gmelin. Syst. Nat., i, 1788, p. 3040 Rudolphi. Entoz. Synops., 1819, p. 7 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 421	*Felis leo*	Under the skin
Syn. *Ascaris leonis*	Gmelin. Syst. Nat., i, 1788, p. 3031		
F. leporis. Gmelin	Gmelin. Syst. Nat., i, 1788, p. 3040 Zeder. Naturg. d. Eingw., 1803, p. 38 Rudolphi. Entoz. Synops., 1819, p. 8 Dujardin. Hist. Nat. d. Helm, 1845, p. 48 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 421	*Lepus timidus*	Thigh and lumbar region
F. bidentata. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 401	*Cervus nambi; C. simplicornis; C. rufus* (Brazil)	Abdominal cavity
F. incrassata. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 389 Diesing. Wien. Sitzber, xlii, p. 700	*Nasua socialis* *Bradypus tridactytus* (Brazil)	
F. inflexo candata. Siebold	Siebold. Wiegmann's Arch., 1842, p. 348 Diesing. Syst. Helm., ii, 1851, p. 281 Baird. Catal. of Entoz. London, 1853, p. 7 Molin. Wien. Sitzber, xxviii, 1858, p. 422 Benedin. Bull. Acad. Roy. Bruxelles Sc., tom. xxix, 1870, p. 364	*Phocaena communis*	Encysted in the lungs
F. insignis. Leidy	Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1858, p. 112 Diesing. Wien. Sitzber, xlii, 1860, p. 711	*Procyon lotor*	Encysted under skin of feet
F. intercostalis. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 418	*Chrysothrix sciurea* (Brazil)	Between the intercostal muscles
F. bifida. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 411	*Dactylomys amblyonyx* (Brazil)	Liver
Syn. *Dicheilonema bifidum*	Diesing. Wien. Sitzber, xlii, 1860, p. 707		
F. nodosa. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 380 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Hapale melanura; Callithrix personata*	Beneath the skin
F. pistillaris. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 381 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Sciurus igniventris* (Brazil)	Beneath the skin
F. annulata. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 386	*Lagothrix cana* (Brazil)	
F. anticlava. Molin	Molin. Wien. Sitzber, xxviii, p. 381 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Dasypus sexcintus*	Stomach
F. acuticauda. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 379	*Dasypus loricatus; D. niger* (Brazil)	Under the skin of the neck
F. aequalis. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 383	*Myrmecophaga jubatu* (Brazil)	
Syn. *Solenonema aequale*	Diesing. Wien. Sitzber, xlii, 1860, p. 704		
F. terminalis. Passerini	Passerini. Atti Soc. Ital. d. sc. nat. Milano, xxviii, 1884, p. 42 Parona. Elmintol. italiana. Genova, 1894, p. 241	*Lepus timidus* (Tuscany)	Lungs
F. oculi canini. Gescheidt	Railliet. Zool. med. et agric. Paris, 1893, p. 531	*Canis familiaris*	Vitreous body of eye
Syn. *F. trispinalosa*	Diesing. Syst. Helm., ii, 1851, p. 274 Molin. Wien. Sitzber, xxviii, 1858, p. 402 Davaine. Traité d. Entoz. Paris, 1877, p. 108		
F. vulpis. Rudolphi	Rudolphi. Entoz. Synops, 1819, p. 7 Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 420	*Canis vulpes*	Abdomen
F. vespertilionis. Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 7 Dujardin. Hist. Nat. d. Helm., 1845, p. 47 Diesing. Syst. Helm., ii, 1851, p. 279 Molin. Wien. Sitzber, xxviii, 1858, p. 419	*Vespertilio discolor* *V. bechsteinii*	Abdomen
F. torta. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 390 Diesing. Wien. Sitzber, xlii, 1860, p. 700	*Lagothrix cana* (Brazil)	
F. macropodis gigantis. Webster	Diesing. Syst. Helm., ii, 1851, p. 280 Molin. Wien. Sitzber, xxviii, 1858, p. 422	*Macropus giganteus*	Knee
F. turdi olivascentis. Molin	Molin. Wien. Sitzber, xlii, 1860, p. 423	*Turdus olivascens* (Brazil)	Beneath the nictitating membrane
F. dubia. Stossich			
Syn. *F. verrucosa*	Molin. Wien. Sitzber, xlii, 1860, p. 392 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Falco swainsonii* (Brazil) *Strix suinda*	Between the muscles of the lower jaw
F. papilloso-annulata. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 399 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Falco swainsonii* (Brazil)	Orbit

Name	Literature	Host	Site
	(*b*) Aves		
F. campanulata. Molin	Molin. Wien. Sitzber, xxviv, 1858, p. 392 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Falco magnirostris* (Brazil)	
F. tendo. Nitzsch		*Falco peregrinus*	
F. labiotruncata. Molin Syn. *Dicheilonema labiotruncatum*	Molin. Wien. Sitzber, xxviii, 1858, p. 412 Diesing. Wien. Sitzber, xlii, 1860, p. 708	*Tinamus adspersus*, *T. strigulosus*, *T. variegatus* (Brazil)	Abdominal cavity, and under skin
F. quadrilabiata. Molin Syn. *Tetracheilonema quadrilabiatum*	Molin. Wien. Sitzber, xlii, 1860, p. 417 Diesing. Wien. Sitzber, xlii, 1860, p. 711	*Tinamus rufescens*, *T. maculosus* (Brazil)	Abdominal cavity, and subcutaneous
F. tinami variegati. Molin	Molin. Wien. Sitzber, xxviii, 1858, p, 427	*Tinamus variegatus* (Brazil)	Beneath the nectitating membrane
F. subspiralis. Diesing	Diesing. Syst. Helm., ii, 1851, p. 268 Molin. Wien. Sitzber, xxviii, 1858, p. 391 Diesing. Wien. Sitzber, xlii, 1860, p. 700	*Ardea cinerea*, *A. leucogaster* (Brazil)	Beneath the skin of the feet
Syn. *F. ardeae cinereae*	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 56		
F. ardeae. Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 428	*Ardea exilis* (Brazil)	Under the tongue
F. myotherae campanisonae. Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 425	*Formicivora campanisona* (Brazil)	Eye
F. myotherae chrysopygae. Molin	Molin. Hist. Nat. d. Helm., 1858, p. 425	*Formicivora chrysopyga* (Brazil)	Under skin near the eyes
F. myotherae regis. Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 424	*Formicivora rex* (Brazil)	Kidney
F. myotherae ruficipitis. Molin	Molin. Hist. Nat. d. Helm, xxviii, 1858, p. 424	*Formicivora ruficeps* (Brazil)	Abdominal cavity
F. tridens. Molin Syn. *F. cassiciatri*	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 393 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 702 Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 423	*Lanius collurio*; *Icterus cristatus*; *I. haemorrhous*; *I. icterocephalus*; *I. chopi*; *I. sericeus*; *Cassicus ater*; *C. viridis*	Abdominal and thoracic cavities
F. icteri pyrrhopteri. Molin	Molin. Hist. Nat. d. Helm., xxviii, p. 423	*Icterus pyrrhopterus* (Brazil)	Abdominal and thoracic cavities
F. bipapillosa. Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 399 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 702	*Strix suinda* (Brazil)	Under the skin of the neck
F. hystrix. Molin	Molin. Hist. Nat. d. Helm., xxviii, 1858, p. 408 Diesing. Hist. Nat. d. Helm., xlii, 1860, p. 703	*Strix flammea* (Brazil)	Abdominal cavity
F. acuta. Diesing	Diesing. Syst. Helm., ii, 1851, p. 277 Molin. Wien. Sitzber, xxviii, 1858, p. 411 Braun. Arch. d. Fr. d. Naturg., i, M., 1891, p. 112	*Podiceps cristatus* (Rostock); *P. cornutus*	Abdominal cavity
Syn. *F. colymbi* *Dicheilonema acutum*	Rudolphi. Entoz. Synops., 1819, p. 10 Diesing. Wien. Sitzber, xlii, 1860, p. 707		
F. subulata. Deslongchamps	Dujardin. Hist. Nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 283 Molin. Wien. Sitzber, xxviii, p. 429	*Podiceps auritus* (Caen)	Abdominal cavity
F. clavato-verrucosa. Molin	Molin. Wien. Sitzber, xxviii, p. 380 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Thamnophilus canadensis* (Brazil)	On the intestine
F. attenuato-verrucosa. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 394 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Thamnophilus canadensis* (Brazil)	Internal body cavity
F. piprae caudatae. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 424	*Pipra caudata* (Brazil)	Abdominal cavity
F. tricoronata. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 400 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Pipra inornata* (Brazil)	Abdominal cavity
F. veneta. Stossich Syn. *F. quadrispina*	Molin. Wien. Sitzber, xxxiii, 1858, p. 301 Molin. Denkschr. Wien. Akad., xix, 1861, p. 318 Diesing. Wien. Sitzber, xliii, 1861, p. 280 Parona. Elmintol. italiana. Genova, p. 243	*Ibis falcinellus* (Padua)	Between the walls of the stomach
F. tantali cayennensis. Molin	Molin. Elmintol. italiana. Genova, p. 435	*Ibis cayennensis* (Brazil)	Stomach wall
F. carduelis. Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 9 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 281 Molin. Wien. Sitzber, xxviii, 1858, p. 423	*Fringilla carduelis*	Thigh
F. affinis. Rudolphi	Rudolphi. Entoz. Synops., 1819, pp. 4 and 209 Dujardin. Hist. Nat. d. Helm., 1845, p. 54 Diesing. Syst. Helm., ii, 1851, p. 268 Molin. Wien. Sitzber, xxviii, 1858, p. 396 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Fringilla hispaniolensis* (Spain)	Abdominal cavity
F. dendrocalaptis procurvi. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 425	*Dendrocalaptes procurvus* (Brazil)	Eye
F. quadriverrucosa. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 398 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Dendrocalaptes picus*; *D. rufirostris* (Brazil)	Abdominal cavity
F. coronata. Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 6 Lamark. Anim. s. Vert., iii, 1840, p. 668 Dujardin. Hist. Nat. d. Helm., 1845, p. 55 Diesing. Syst. Helm., ii, 1851, p. 275 Molin. Wien. Sitzber, xxviii, 1858, p. 408, and xxx, 1858, p. 155 Diesing. Wien. Sitzber, xlii, 1860, p 703; xliii, 1861, p. 280	*Coracias garrula* (Padua, Vienna, Turkestan)	Under the muscles of head—and skin of neck

Name	Literature	Host	Site
F. coronata. Rudolphi—contd.	Linstow. Arch. f. Naturg., xlix, 1883, p. 286 ; Vermi, Mosca, 1886, p. 11		
	Parona. Elmintol italiana. Genova, 1894, p. 242		
Syn. *Ascaris coraciae*	Gmelin. Syst. Nat., p. 3033		
Fusaria coraciae	Zeder. Naturg. d. Eingw., 1803, p. 119		
F. obtusa. Leidy.	Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1886, p. 308	*Junco hyemalis*	
Syn. *F. obtusa*	Leidy. Proc. Acad. Nat. Sc. Philadelphia, 1885, p. 10	*Sturnella magna*	
F. sturni. Rudolphi.	Rudolphi. Entoz. Synops., 1819, p. 9	*Sturnus vulgaris*	Lungs and thoracic cavity
	Dujardin. Hist. Nat. d. Helm., 1845, p. 53		
	Diesing. Syst. Helm, ii, 1851, p. 281		
	Molin. Wien. Sitzber, xxviii, 1858, p. 424		
F. abbreviata. Rudolphi	Rudolphi. Entoz. Synops., 1819, pp. 4 and 210	*Saxicola sp.* (Turkestan) ;	The internal cavities of the body
	Dujardin. Hist. Nat. d. Helm., 1845, p. 52	*Luscinia philomela ;*	
	Diesing. Syst. Helm., ii, 1851, p. 268	*Motacilla melanocephala*	
	Molin. Wien. Sitzber, xxviii, 1858, p. 396	(Brazil) ; *Saxicola oenanthe ;*	
	Linstow. Arch. f. Naturg., xlix, 1883, p. 286	*S. stapezina ; Luscinia*	
	Linstow. Vermi. Mosca, 1886, p. 11	*rubecula ; Turdus pilaris ;*	
Syn. *F. motacillae*	Rudolphi. Entoz. Synops., 1819, p. 635	*T. visciverus ; Sturnus*	
F. motacillarum	Rudolphi. Entoz. Synops., 1819, p. 9	*pyrrhocephalus* (Brazil) ;	
F. turdorum	Rudolphi. Entoz. Synops, 1819, p. 9	*Tanagra jacapa* (Brazil) ;	
F. philomelae	Diesing. Syst. Helm., ii, 1851, p. 226	*Thryothorus polyglottus*	
		(Brazil) ; *Furnarius rufus*	
		(Brazil) ; *F. leucops* (Brazil)	
F. simplicissima. Molin	Molin. Syst. Helm., xxviii, 1858, p. 372	*Psittacus makaonanna* (Brazil)	Beneath the skin
	Diesing. Syst. Helm., xlii, 1860, p. 701		
F. truncato-caudata. Deslongchamps	Dujardin. Hist. Nat. d. Helm., 1845, p. 56	*Vanellus cristatus* (Caen, Turkestan)	Abdominal cavity
	Diesing. Syst. Helm., ii, 1851, p. 283		
	Molin. Wien. Sitzber, xxviii, 1858, p. 427		
	Linstow. Arch. f. Naturg., 1883, xlix, p. 287		
	Linstow. Vermi. Mosca, 1886, p. 11		
F. bifurca. Molin	Molin. Wien. Sitzber, xviii, 1858, p. 400	*Muscicapa sp.* (Brazil)	Abdomen
F. bilabiata. Diesing	Diesing. Syst. Helm., ii, 1851, p. 277	*Sterna leucopareia*	Abdominal cavity
	Molin. Wien. Sitzber, xxviii, 1858, p. 411		
Syn. *F. sternae*	Rudolphi. Entoz. Synops, 1819, p. 10		
Dicheilonema bilabiatum	Diesing. Wien. Sitzber, xlii, 1860, p. 707		
F. cyngi. Rudolphi	Rudolphi. Entoz. Synops., 1819, p. 10	*Cygnus musicus*	Abdominal cavity
	Dujardin. Hist. Nat. d. Helm., 1845, p. 58		
	Diesing. Syst. Helm., ii, 1851, p. 284		
	Molin. Wien. Sitzber, xxviii, 1858, p. 429		
	Railliet. Zool. med. et Agric. Paris, 1893, p. 533		
	Parona. Elmintol. italiana. Genova, 1894, p. 243		
Syn. *Ascaris cygni*	Gmelin. Syst. Nat., p. 3033		
Fusaria cygni	Zeder. Naturg. d. Eingw., 1803, p. 119		
F. armata. Gescheidt	Diesing. Syst. Helm., ii, 1851, p. 275	*Buteo lagopus*	In vitreous humour of the eye
	Molin. Wien. Sitzber, xxviii, 1858, p. 404		
	Diesing. Wien. Sitzber, xlii, 1860, p. 703		
F. anatis. Rudolphi	Rudolphi. Entoz. Synops, 1819, p. 20	*Anas domestica*	The leg
	Dujardin. Hist. Nat. d. Helm., 1845, p. 58		
	Diesing. Syst. Helm., ii, 1851, p. 284		
	Molin. Wien. Sitzber, xxviii, 1858, p. 429		
	Railliet. Zool. med. et agric. Paris, 1893, p. 533		
F. sylviae. Nordmann	Nordmann. Microgr. Beiträge, i, 1832, p. 17	*Sylvia abietina*	Orbit
	Diesing. Syst. Helm., ii, 1851, p. 281		
	Molin. Wien. Sitzber, xxviii, 1858, p. 423		
F. spaerophora. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 401	*Anabates anthoides ; Muscicapa lophotes* (Brazil)	Liver
F. spinulosa. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 350	*Glareola austriaca*	Stomach wall
	Diesing. Wien. Sitzber, xlii, 1860, p. 712		
F. trochili amethystini. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 426	*Calliphlox amethystina* (Brazil)	On the stomach
F. lari. Rudolphi	Rudolphi. Entoz. Synops, 1819, pp. 10 and 218	*Larus minutus* (Vienna)	Under the skin of the neck
	Dujardin. Hist. Nat. d. Helm., 1845, p. 58		
	Diesing. Syst. Helm., ii, 1851, p. 283		
	Molin. Wien. Sitzber, xxviii, 1858, p. 429		
F. meropis. M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 9	*Merops apiaster*	Mesentery
	Dujardin. Hist. Nat. d. Helm., 1845, p. 55		
	Diesing. Syst. Helm., ii, 1851, p. 281		
	Molin. Wien. Sitzber, xxviii, 1858, p. 426		
F. aspera. Nitzsch		*Otis brachyotus*	Subcutaneous
F. podoae. Molin	Molin. Wien. Sitzber, xxviii, 1818, p. 428	*Podoa surinanemsis* (Brazil)	Beneath the skin of the neck
F. charadrii. M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 10	*Charadrius fluviatilis* (Vienna)	Under the skin of the nose and ear
	Dujardin. Hist. Nat. d. Helm., 1845, p. 56		
	Diesing. Syst. Helm., ii, 1851, p. 283		
	Molin. Wien. Sitzber, xxviii, 1858, p. 427		
F. circumflexa. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 377	*Trogon aurantius* (Brazil)	Abdominal cavity

Name	Literature	Host	Site
F. tringae. M.C.V.	Rudolphi. Entoz. Synops., 1819, p. 10 Diesing. Syst. Helm., 1851, p. 282	*Tringa variabilis*	Beneath the skin
F. serotina. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 374 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Lichenops perspicillata* (Brazil)	Abdominal cavity
F. fusiformis. Molin Syn. *Dicheilonema fusiforme*	Molin. Wien. Sitzber, xxviii, 1858, p. 415 Diesing. Wien. Sitzber, xlii, 1860, p. 709	*Monasa tranquilla* (Brazil)	Thoracic cavity
F. physalura. Bremser	Diesing. Syst. Helm., ii, 1851, p. 256 Molin. Wien. Sitzber, xxviii, 1858, p. 412 Leidy. Proc. Acad. Nat. Sc, Philadelphia, 1885, p. 10	*Alcedo amazona; A. torquati, A. supercilosa; Ceryle alcyon* (Brazil)	Abdominal cavity
Syn. *F. alcedinus*	Rudolphi. Entoz. Synops., 1819, p. 635		
F. alcedinus superciliosae	Molin. Wien. Sitzber, xxviii, 1858, p. 426		
Monopetalonema physalurum	Diesing. Wien. Sitzber, xlii, 1860, p. 710		
F. corvi torquati. Cobbold and Manson		*Corvus torquati*	Right ventricle of heart and pulmonary
F. picae mediae. Cobbold and Manson		*Pica media*	In tubercles on the pulmonary and aortic valves
F. helicina. Molin.	Molin. Wien. Sitzber, xxviii, 1858, p. 391	*Plotus anhinga* (Florida)	Brain
F. hemicyla. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 377 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Pionus menstruus* (Brazil)	Under the skin of the neck
F. bonasiae. Nordmann	Dujardin. Hist. Nat. d. Helm., 1845, p. 56 Diesing. Syst. Helm., ii, 1851, p. 282 Molin. Wien. Sitzber, xxviii, 1856, p. 426	*Tetrao bonasio*	Eye
F. perdicis dentatae. Molin.	Molin. Wien. Sitzber, xxviii, 1858, p. 427	*Perdix dentata* (Brazil)	Abdominal cavity
F. triaenucha. Wright	Wright. Americ. Helminth I, 1879, p. 21	*Botaurus minor* (America)	Proventicule
F. dipetala. Molin Syn. *Dipeta lonema inflexum*	Molin. Wien. Sitzber, xxviii, 1858, p. 373 Diesing. Wien. Sitzber, xlii, 1860, p. 704	*Platyrhynchus petangua* (Brazil)	Abdominal cavity
F. cirrura. Leidy	Leidy. Proc. Acad. Nat. Sc. Philadelph. viii, 1886, p. 309	*Guiscalus major* (Florida)	

(*c*) Reptilia

F. mucronata. Molin	Molin. Wien. Sitzber, xxx, 1858, p. 155 Molin. Denkschr. Wien. Akad., xix, 1861, p. 318		
Syn. *Dipetalonema mucronatum*	Diesing. Wien. Sitzber, xlii, 1860, p. 704, and xliii, 1861, p. 280	*Boa constrictor*	Thoracic cavity
F. bispinosa. Diesing	Diesing. Syst. Helm., ii, 1851, p. 278 Leidy. Proc. Acad. Nat. Sc. Philadelphia, viii, 1856, p. 56 Diesing. Denkschr. Wien. Akad., xiii, 1879, p. 18 Molin. Wien. Sitzber, xxviii, 1858, p. 415	*Boa constrictor; Ophis saurocephalus; Thamnobius poecitostomo* (Brazil)	Under the skin, in the abdominal cavity, and in the walls of the oesophagus and intestine
Syn. *Dicheilonema bispinosum*	Diesing. Wien. Sitzber, xlii, 1860, p. 709		
F. boae constrictoris	Leidy. Proc. Acad. Nat. Sc. Philadelphia, v, 1851, p. 118		
F. megalochila. Diesing	Diesing. Syst. Helm., ii, 1851, p. 278 Molin. Wien. Sitzber, xxviii, 1858, p. 417	*Coronella austriaca*	Oesophagus
Syn. *F. colubri anstriaci*	Rudolphi. Entoz. Synops, 1819, p. 10		
Tricheilonema megalochilum	Diesing. Wien. Sitzber, xlii, 1860, p. 711		
F. podinemae sciptae. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 430	*Podinema scipta* (Brazil)	In the abdominal fat
F. haje. Wedl.	Wedl. Wien. Sitzber, xliv, 1861, p. 472	*Naja haje*	All parts external to lungs
F. hebetata. Cobbold		*Cystophora cristata*	Right heart
Filaria bacillaris. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 379 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Caiman niger; C. sclerops* (Brazil)	Lungs
F. cloeliae fasciatae. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 431	*Oxyrhopus fasciatis* (Brazil)	Encysted in stomach wall
F. colubri. Box	Box. Hist. Nat. d. vers. Paris, ii, 1802, p. 49 Dujardin. Hist. nat. d. Helm., 1845, p. 58 Diesing. Syst. Helm., ii, 1851, p. 284 Molin. Wien. Sitzber, xxviii, 1858, p. 431	*Coluber sp.* (America)	Intestine
Syn. *F. colubri americani*	Rudolphi. Entoz. Synops., 1819, p. 10		
F. colubri aenei. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 435	*Helicops carinicandus*	Abdominal cavity
F. multipapilla. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 385 Diesing. Wien. Sitzber, xlii, 1860, p. 700	*Thorictis dracaena; Iguana tuberculata* (Brazil)	Abdominal cavity
F. calcarata. Molin.	Molin. Wien. Sitzber, xxviii, 1858, p. 378 Diesing. Wien. Sitzber, xlii, 1860, p. 701	*Bothrops jararacca* (Brazil)	Abdominal cavity
F. eunectis scytalis. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 430	*Eunectes scytale* (Brazil)	Lungs
F. solitaria. Leidy	Leidy. Proc. Acad. Nat.Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber, xxviii, 1858, p. 430 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Emys serrata; Chelonura serpentina* (Georgia)	Wall of stomach
F. cistudinis. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber, xxviii, 1858, p. 430	*Cistudo carolina* (America)	Heart

(*d*) Amphibia

Name	Literature	Host	Site
F. rubella. Rudolphi	Rudolphi. Entoz. Synops., 1819, pp. 5 and 212 Dujardin. Hist. Nat. d. Helm., 1845, p. 59 Diesing. Syst. Helm., ii, 1851, p. 269 Molin. Wien. Sitzber, xxviii, 1858, p. 372	*Rana esculenta* (Berlin)	Mesentery; stomach and intestinal walls
Syn. *F. ranae esculentae*	Valentin. Wiegmann's Arch., 1842, p. 312 Diesing. Syst. Helm., ii, 1851, p. 284 Molin. Wien. Sitzber, xxviii, 1858, p. 431		
F. neglecta. Diesing	Diesing. Syst. Helm., ii, 1851, p. 276 Molin. Wien. Sitzber, xxviii, 1858, p. 409 Diesing. Wien. Sitzber, xlii, 1860, p. 703	*Rana esculenta*	Under the skin
Syn. *ranae esculentae*	Rudolphi. Entoz. Synops, 1819, p. 10		
F. nitida. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber, xxviii, 1858, p. 378	*Rana pipiens* (America)	Encysted on the peritoneum and abdominal muscles
F. convoluta. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 390 Diesing. Wien. Sitzber, xlii, 1860, p. 702	*Cystignatus gigas*, *Leptidactylus sibilatrix* (Brazil)	Abdomen
F. amphiumae. Leidy	Leidy. Proc. Acad. Nat. d. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber, xxviii, 1858, p. 431	*Amphiuma meaus* (Philadelphia)	In stomach wall
F. cupemphigis marmorati. Molin	Molin. Wien. Sitzber, xxviii, 1858, p. 431	*Eupemphix marmoratus* (Brazil)	Abdominal cavity

(*e*) Pisces

Name	Literature	Host	Site
F. triglae. Bellingham	Bellingham. Ann. of Nat. Hist., xiv, 1844, p. 475 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber, xxviii, 1858, p. 432	*Trigla cuculus* (Ireland)	Peritoneum
F. quadrituberculata. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien, Sitzber, xxviii, 1858, p. 410	*Anguilla vulgaris* (America)	Dorsal muscles
F. mugilis. Bellingham	Bellingham. Ann. of Nat. Hist., xiv, 1844, p. 475 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber, xxviii, 1858, p. 433	*Mugil capito* (Ireland)	Peritoneum
F. ranae. M.C.V.	Molin. Wien. Sitzber, xxviii, 1858, p. 431	*Hypsiboas faber* (Brazil)	Intestine
F. rubra. Leidy	Leidy. Proc. Acad. Nat. Sc., Philadelphia, viii, 1856, p. 56 Molin. Wien. Sitzber, xxviii, 1858, p. 415	*Labrax lineatus* (America)	Peritoneum
Syn. *Dicheilonema rubrum*	Diesing. Wien. Sitzber, xlii, 1860, p. 708		
F. crassiuscula. Nordmann.	Nordmann. Microgr. Beiträge, 1832, p. 20 Dujardin. Hist. Nat. d. Helm., 1845, p. 62 Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber, xxviii, 1858, p. 433	*Gadus aeglefinis*	Eye
F. extenuata. Deslongchamps	Dujardin. Hist. Nat. d. Helm., 1845, p. 61 Diesing. Syst. Helm., ii, 1811, p. 285 Molin. Wien. Sitzber, xxviii, 1858, p. 432	*Mullus surmuletus* (Caen)	Abdomen

(*f*) Coelenerata

Name	Literature	Host	Site
F. loliginis. Delle Chiaje	Diesing. Syst. Helm., ii, 1851, p. 286 Molin. Wien. Sitzber, xxviii, 1858 p. 434 Parona. Elmintol. italiana. Genova, 1894, p. 244	*Loligo vulgaris* (Naples)	
F. succineae. Siebold	Siebold. Wiegmann's Arch., 1837, p. 255 Diesing. Syst. Helm., ii, 1851, p. 287 Molin. Wien. Sitzber, xxviii, 1858, p. 434	*Succinea amphibia*	Abdomen

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THE HIBERNATION OF MOSQUITOES

THE HIBERNATION OF ENGLISH MOSQUITOES*

BY

H. E. ANNETT, M.D., AND J. EVERETT DUTTON, M.B.

In the *British Medical Journal* of April 27, 1901, Dr. M. J. WRIGHT records some interesting experiments concerning the resistance of mosquito larvae to cold. It is truly a remarkable feature that larvae, both *Culex* and *Anopheles*, are able to withstand a temperature of about 4° C. for a period of two weeks.

Early in December last Mr. T. V. THEOBALD, of Wye, Kent, sent a number of *Anopheles* larvae to Major Ross, which have been kept continuously in a greenhouse at a temperature of from 15° C. to 32° C., and, although they are often seen feeding on the green protococcal growth supplied, they show apparently very little increase in size, and none have as yet changed † into pupae.

Dr. WRIGHT infers from his observations that the larval form is that in which 'hibernation' takes place; never having found adult mosquitoes during the winter months. Here his results differ most markedly from ours, which we wish now to record in a short preliminary account. It may be mentioned that throughout the winter session a supply of *Culex* adults has been obtainable for class purposes at this School.

On February 17, during a period of very cold weather, four *Anopheles* were caught at a farm some thirteen miles from Liverpool, in North Cheshire. A large number of *Culex* (four species) were also captured here. The *Anopheles* were identified by Mr. T. V. THEOBALD as *A. maculipennis*. The mosquitoes were found in the following situations: cellar, dairy, cheese room, pantries, lumber rooms, and in some disused bedrooms at the top of the house; also in the wash-house and whey tank house abutting on to the house, and in the coach-house, tool sheds, and privies at some distance away. No mosquitoes could be found in the stables, cowsheds, pigsties, haylofts, henpens. Many of the farm houses of this district of Cheshire are old, and have no damp-proof courses. In the disused cellars the walls and the beams supporting the ground floor were soaking with moisture, and small ferns grew in the crevices of the tiled floor, and patches of moss and mould on the surface of the walls. Here thousands of mosquitoes, chiefly *Culex*, blackened the walls and rafters. In the dairies they were found on the damp areas, resting on and in the crevices of the plaster; very few were seen on the drier parts. Similarly in the other places, on the damp portions, many mosquitoes were observed, especially behind boxes, slates, boards, barrels, and other articles resting against the wall.

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† April 16, 1901.

Since the date* mentioned, *Anopheles* have been collected on four occasions from farms in North and Mid Cheshire. The Mid Cheshire farm is at a distance of about thirty-five miles from Liverpool, and here *Anopheles maculipennis* occurred in about the same number and in similar situations as in the North Cheshire farms. About twenty *Anopheles* were collected at each farm. In all these sites it was noted that the majority of the mosquitoes of the genus *Culex* were found on the parts of the damp walls near the ground, while *Anopheles* were generally caught near the ceiling. During the coldest weather the attitude both of *Culex* and *Anopheles* was peculiar and characteristic. The under surface of the thorax and abdomen was applied closely to the wall, while the legs were stretched straight out almost at right angles to the body. The absence of the characteristic attitude of *Anopheles* (at an angle to the surface), and the fact that both *Culex* and *Anopheles* assumed the peculiar outstretched attitude, made it difficult at first sight to distinguish specimens of the two genera, especially since among the *Culex* were a species having wings spotted somewhat similarly to *Anopheles* (*Culex annulatus*): but on closer inspection even in the position described, the characteristic angle, seen in side view, between the direction of the head and thorax and of the abdomen of *Culex*, served to distinguish the genera. In this peculiar attitude the mosquitoes were very difficult to rouse; the mouth of a bottle could be easily placed over them without disturbing them, and in fact, one had to lift them on to their legs by the rim, and then no attempt was made to fly: they would crawl lazily along the neck of the bottle. How long these mosquitoes remain in this position during the winter months is not easy to determine, but it was noticed that many of the *Culex* on the damper patches were wholly or partially enveloped in a thick mould which had grown in and around their bodies, thus fixing them in the attitude described. On very cold days this attitude was observed even in the bottles in which the mosquitoes had been collected. On warmer days at the farms, and on taking the bottled mosquitoes into a warm room, they assumed their ordinary attitudes.

It has been mentioned above that mosquitoes were not found, or only very rarely, in stables, pigsties, and henpens, etc., which were frequented by animals. Such places are generally comparatively dry, constantly disturbed, and warmed by the presence of horses and cattle at night.

A number of the *Anopheles* collected by us have been kept in a damp cage in the animal house of the Thompson Yates laboratories, no food having been supplied; only two of the number have died during the month we have kept them in this condition. There can, therefore, be no doubt that mosquitoes of both genera 'hibernate' during the winter months in England, and it seems certain that not only the adults but, from Dr. WRIGHT's experiments, the larval forms also provide for the continuation of the species during the cold weather.

It is of interest to note that among the numbers of mosquites of both genera collected by us a male was never found; and, moreover, that all the females with

which we carried on a number of experiments or which we dissected had been fertilized (proved by the presence of spermatozoa in the spermatheca, or by the hatching out of larvae from deposited eggs).

A number of experiments relating to the bionomics of the English *Anopheles maculipennis* are at present being undertaken, and we hope to be able to communicate the detailed results at a later date. Some interesting facts may, however, be recorded. If these mosquitoes be kept in a *dry* cage they die in a few days—whereas, as stated above, they can be kept probably for months in a *damp* cage in the cold, during which time they preserve, what we propose to call, the 'hibernating' attitude. On introducing them into a warm room they quickly become active, and both *Anopheles* and some species of *Culex* eagerly feed on blood on inserting the hand into the cage, darkened by covering with a cloth. They then feed eagerly every day for four or five days, but subsequently only occasionally. Eggs were laid on the fifth, sixth, seventh, and eighth days, which hatched out in twenty-four or forty-eight hours. It was noted that many of the *Anopheles* died after depositing a batch of eggs.

We have further observed that having once fed on blood, it is necessary to continue the feedings at least every other day, otherwise the ovaries cease to develop and the insects die, though water is supplied. This confirms our experiments made in West Africa,* where by regular feeding we were able to keep *Anopheles costalis* and *funestus* alive for a considerable period; while in the present case of *A. maculipennis* which had been hibernating, most of them died soon after laying eggs.

On the 19th April* of this year, during a period of about two weeks of fine warm weather, we made another visit to the farms at which we had on previous occasions never failed to collect *Anopheles*. At one farm, three or four *Anopheles maculipennis* were seen, but being so very active only two were caught. At another some fifty were seen, but only ten caught, and these with great difficulty. The mosquitoes were exceedingly active, flying immediately the light of the candle fell upon them, and directly the bottle was placed near them. This activity strikingly contrasted with their slow, lazy movements of the previous week during colder weather. Many hundreds of *Culex* of different species were seen, their increased activity was also noticeable.

On the 23rd of the same month* another visit was made to these farms, but no specimen of *Anopheles* was seen in the sites where previously so many had been captured. At the same time other farms up to now not visited were examined without success. At one farm, however, we were allowed to search the whole house, and here found five specimens of *Anopheles maculipennis*, gorged with blood and showing developing ovaries, in the attics in which several Irish farm labourers slept,

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* *Report of the Liverpool Malaria Expedition to the Nigeria*, 1901, p. 37-45

on the ceiling of the staircase, and on the ceiling and walls of the kitchen. In the other less-used and more cleanly-kept rooms none could be found. The visit was made about mid-day.

On the 5th of May,* at a farm near Chester, one female and two males, *Anopheles bifurcatus*, were captured in the laundry in the dusk of the evening. An examination of other parts of the farm was unsuccessful, except in the cellar, where the crowd of *Culex* was found to have left the wall, and had collected near the only possible exit (a fine wire grating of perforated zinc in the wall) where myriads had been killed in their endeavours to escape.

The *Anopheles* caught on the 23rd of April* we firmly believe to have hibernated during the winter months, and at that time were developing ova for the first time this year, having frequently during the winter and early spring months examined many farms in this district.

* The figures below give the mean daily temperature for each week throughout March, April, and May, in this district.

For week ending	March	7	- - - -	42·3° F.
„ „	„	14	- - - -	43·1° F.
„ „	„	21	- - - -	38·4° F.
„ „	„	28	- - - -	35·8° F.
„ „	April	4	- - - -	41·5° F.
„ „	„	11	- - - -	45·2° F.
„ „	„	18	- - - -	44·2° F.
„ „	„	25	- . - -	57·0° F.
„ „	May	2	- - - -	49·5° F.
„ „	„	9	- - - -	49·9° F.
„ „	„	16	- - - -	52·1 F.
„ „	„	23	- - - -	54·8° F.
„ „	„	31 (8 days)	- - -	58·4° F.

THE FLORA OF THE CONJUNCTIVA IN HEALTH AND DISEASE

THE FLORA OF THE CONJUNCTIVA IN HEALTH AND DISEASE *

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Introduction

The large and varied supply of ophthalmic material available in the eye clinic of the Royal Infirmary and in the Parish Infirmary of Liverpool, has afforded me the opportunity for the work embodied in my thesis.

I should like at the outset to express my thanks, in the first place, to Dr. Alexander, the visiting surgeon of the Parish Infirmary, from whose wards I have obtained the bulk of my material, and also to Mr. Bickerton, Ophthalmic Surgeon to the Royal Infirmary.

The research has been conducted with the following objects in view :—

(*a*) To determine the flora of the normal conjunctival sac.

(*b*) To compare the pathogenic properties of organisms occurring in healthy eyes with similar organisms found in diseased eyes.

(*c*) To investigate the causal agents of the various suppurative inflammations of the conjunctiva met with.

The first part is a record of the results obtained in the bacteriological investigation of a number of healthy and diseased conjunctival sacs with a tabulated series of experiments on rabbits' eyes with some of the principal organisms isolated.

Further, to determine whether pyogenic cocci could be found in the normal conjunctival sac and in how far they possessed virulent properties as compared with similar organisms found in diseased eyes.

Apart from its scientific interest such an investigation is of the greatest importance to the ophthalmic surgeon not only in operative procedures but also in elucidating obscure points in the etiology of conjunctival disease.

In each case examined all the organisms cultivated have been noted and, for those that could not be named, a brief account of the principal cultural features has been given.

Special prominence has been given to the numerous varieties of bacilli resembling the diphtheria bacillus which occur in the conjunctival sac, and which correspond in a marked way to the different forms of diphtheria bacillus isolated by recent observers from the throat and nasal cavities.

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* Presented in the form of a Dissertation to the Victoria University for the Degree of M.D. 1901.

SYNOPSIS OF CONTENTS

Part I

THE NORMAL FLORA

The results given in this section are based upon an examination of 210 cases, in which the conjunctiva was apparently healthy. The material for this purpose has been collected chiefly in the out-patient department of the Liverpool Royal Infirmary from persons suffering from some refractive error or other disease not affecting the conjunctival mucous membrane. A few of the cases were taken from amongst my colleagues in the laboratory, and from the wards of the Liverpool Parish Infirmary, these latter being mainly children.

In collecting this series an endeavour has been made to include individuals differing as much as possible in age, sex, social position, and environment.

Whilst it is obvious that the number and character of the organisms temporarily inhabiting the conjunctival sac will depend largely upon the number of bacteria in the immediate neighbourhood of the individual, a more potent cause of contamination will be found in neglect of personal cleanliness. Dirty fingers, face, etc., are more likely to infect the conjunctiva with pyogenic cocci than the atmosphere, and these cocci will in all probability possess more virulent properties. It might be expected that the number of pyogenic cocci would vary in inverse ratio to the cleanliness of the individual and his surroundings. My cases have shown this expectation to be well founded.

Great care has been exercised in the selection of the cases. All those giving a history of 'gumming' of the lids in the morning, or sensation of grit in the eyes, etc., being discarded, as well as those showing abnormal redness or other apparent sign of disease.

The method adopted in each case was as follows :—The lower lid was everted, and the conjunctiva gently stroked with the loop of a sterilized platinum wire, until the loop had become charged with lachrymal fluid. A serum tube was inoculated by smearing the fluid evenly over the surface, and incubated at 38°C.

It was occasionally very difficult to obtain even a loopful of lachrymal fluid, but slight mechanical stimulation generally sufficed to produce a free flow of tears. The platinum loop was used principally on account of its convenience, and because it was thought that the organisms in the sample would be sufficiently representative of the total bacteriology of the sac.

In some of the cases a sterile cotton-wool swab was used. With this the whole of the lower conjunctival fornix was swabbed out, and after being rubbed well over the surface of serum the swab was placed in broth.

Although this method may remove from the sac a greater number and variety of organisms, it is open to some objection in that in the operation of smearing over

the surface of the medium many organisms will remain entangled in the wool, and in the subsequent broth culture they will either not grow or be overgrown by other organisms to which broth is a more suitable medium.

It has been noted in many cases that a far larger number of colonies, particularly of the xerosis bacillus, has been produced by the use of the loop than by the use of the swab.

Solidified horse-serum was used throughout for the primary inoculations ; in previous investigations it was observed that some of the organisms, notably the xerosis bacillus, present in the sac grew with great difficulty in primary culture on agar-agar, gelatine, etc.

After inoculation the tubes were generally kept in the incubator for forty-eight hours before examination ; at the end of twenty-four hours it was often impossible to detect any growth with the naked eye, whilst at forty-eight hours an abundant growth was revealed. Even those organisms which from other situations presented a good growth at twenty-four hours here produced a very inconsiderable growth.

The loopful of fluid was smeared well over the surface of the serum and the resulting colonies were so perfectly discrete that pure cultures of the different organisms could easily be obtained.

The following table shows the source of the material and the percentage of sterile sacs in the different groups of individuals.

TABLE I

No.	Source	No. of Sacs examined	Sterile Tubes	Method	Percentage Sterile
1	Workers in Laboratory	12	8	Platinum loop	66·6
2	Liverpool Parish Infirmary (Children)	40	11	Platinum loop and Diphtheria swab	27·5
3	Liverpool Royal Infirmary				
	(*a*) Children and Adults	146	25	Platinum loop	17·1
	(*b*) Adults	12	3	Diphtheria swab	25·0
	Total	210	47		

TABLE II

Organisms found in the Normal Conjunctival Sac

Xerosis bacillus (Table IV, No. 1)	120 times
Staphylococcus epidermidis albus (Welch)	47 ,,
Staphylococcus pyogenes aureus	8 ,,
Staphylococcus pyogenes citreus	1 ,,
Staphylococcus pyogenes albus	14 ,,
Staphylococcus cereus flavus	1 ,,
Staphylococcus cereus albus	2 ,,
Streptococcus pyogenes longus	8 ,,
Streptococcus brevis...	12 ,,
Pneumococcus (Fraenkel)	2 ,,
Bacillus lacunatus (Eyre)	9 ,,
Bacillus mesentericus ruber	2 ,,
Bacillus subtilis	1 ,,
Bacillus capsulatus mucosus	1 ,,
Bacillus coli communis	1 ,,
Bacillus striatus flavus	2 ,,
'Red bacillus'	2 ,,
Sarcina lutea	3 ,,
Micrococcus tetragenus	1 ,,
Proteus vulgaris	1 ,,
Penicillium glaucum	1 ,,
Cladothrix (white)	1 ,,
Cladothrix (brown)	1 ,,
Table III, No. 1	3 ,,
Table III, No. 2	1 ,,
Table III, No. 4	1 ,,
Table III, No. 5	1 ,,
Table III, No. 6	2 ,,
Table IV, No. 2	1 ,,
Table IV, No. 3	2 ,,

Only forty-seven out of the total number of sacs examined were sterile.

The xerosis bacillus and bacilli of the diphtheria group were found one hundred and twenty-three times, fifty-two times in pure culture, twenty-four associated with the staphylococcus epidermidis albus and forty-seven in mixed culture.

The frequency with which this bacillus occurred suggested that with a more complete examination many of the sterile sacs would have shown the presence of one or two organisms.

It was impossible to make such a systematic examination of all the sterile eyes. In a few cases, however, a number of tubes were inoculated from eyes which had been sterile to one examination, and usually in one of the tubes a single colony of xerosis bacillus or staphylococcus epidermidis albus was found. It is not unlikely

that at the time of examination some conjunctival sacs are absolutely free from organisms, but the frequency of the occurrence of the xerosis bacillus and its presence, or the presence of other organisms, after repeated examination makes the conclusion that the normal conjunctival sac is sterile only in a very small number of cases apparently inevitable.

The list of organisms show that the pathogenic bacteria do not occur very frequently, and the experiments with them on animals demonstrate that they have to a certain extent lost their virulence.

It seems, therefore, that although a conjunctiva is but rarely sterile, the organisms it usually contains would have little effect in prejudicing the result of an aseptic operation.

Certain operations on the eye are performed in the upper quadrants, and, as EYRE[4] pointed out, the upper fornix conjunctivae seldom contains organisms, the frequent sterility of this part of the conjunctiva and the infrequent occurrence of pathogenic organisms explains why eye operations so rarely become septic.

Staphylococcus aureus was isolated eight times—in six instances from children, and only two from adult sacs; the latter were found to be considerably less pathogenic than some of those isolated from children's eyes.

Streptococcus brevis was not found once in adults, the streptococcus longus only twice, and in one of the two was not pathogenic to mice.

The frequent occurrence of both varieties of streptococcus in children's eyes was very striking, and in order to confirm the results twelve additional cases were examined; in these streptococcus brevis occurred four times, streptococcus pyogenes longus twice.

To produce quickly a quantity of streptococcus longus the following method was adopted:—Over a twenty-four hours' sub-culture on slant agar, sterile broth was poured sufficient to cover all the colonies, and incubated at 38° C.; in one day each colony had grown out into the broth as a delicate villous-like prominence, with the position of the colony as a base; further incubation produced more growth, and if the tube was kept perfectly motionless fairly long threads grew out into the broth; from these growths very beautiful microscopical specimens of chains could be made. The slightest movement of the medium precipitated the mass to the bottom of the tube.

In two cases a few transparent colonies were noticed, which consisted of oval cocci, morphologically similar to the pneumococcus; they quickly died out, and it was not possible to study their life history.

Staphylococcus epidermidis albus (WELCH) was observed on forty-seven occasions. RANDOLPH isolated it in eighty-five out of one hundred cases examined.

The 'red bacillus' resembled in biological characters B. latericeus. On all media it formed a brilliant red growth; on gelatine it grew abundantly, but did not liquefy the medium.

	which spreads slowly and develops a brownish-yellow centre. The surface is dry and glazed. —— *Streak.*—Growth occurs when planted thickly as a flat, raised, translucent, slowly-extending growth with a light-brownish centre. The surface is dry and slightly wrinkled or striated. The edges are crenate. No liquefaction.	ber of opaque, light-yellow colonies.		transparent and feathery. The edges are crenate. —— *Stab.*—In the depth a number of minute, opaque, white colonies. On the surface is a white growth, extending laterally in a number of short, fern-like off-shoots.	well-defined margins. In a week the growth has become a brilliant flesh-colour. —— *Stab.*—Opaque, cream or flesh-coloured expansion on the surface. In the depth thick granular line in which after a time large whitish colonies with a brown nucleus develop.	ing a little laterally with a tendency to heaping in the centre. Margins finely serrate. Later the centre of the growth assumes a wrinkled aspect. —— *Glucose gelatine shake*— No gas formation.
Urine	No ammoniacal decomposition.					
Motility	Non-motile.		Non-motile.	Non-motile.	Non-motile.	Motile.
Staining Reactions	Early cultures stain well with the aniline dyes; old cultures take the stain badly. They do not retain the stain by Gram's method.	Stains well with all the aniline dyes. Stains by Gram's method.	Does not stain by Gram's method.	Stains with all the aniline dyes. Does not retain the stain by Gram's method.	Stains with aniline dyes, and by Gram's method.	Stains with aniline dyes. Does not stain by Gram's method.

No. 3 in Table III was a slender spore bearing bacillus corresponding to the description of the bacillus of COLOMIATTI; morphologically it resembles B. alvei, which was found on one occasion by EYRE in the normal conjunctival sac. The bacillus occurred in muco-purulent catarrh, but for convenience of description I have included it in the description of organisms obtained from healthy eyes.

Many varieties of bacilli resembling in some particular the B. diphtheriae have been isolated from the conjunctiva, and forms varying from a short regular bacillus not forming typical involution forms and a form indistinguishable from the diphtheria bacillus in all its reactions have been obtained.

A table (IV) has been drawn up describing the cultural peculiarities of a few of those bacilli which have features sufficiently different to distinguish them from other members of the group. In this table, for convenience of description and comparison, has been included two varieties (4 and 5) only noticed in pathological conditions.

The organism known as the xerosis bacillus is the most common inhabitant of the conjunctival sac.

In two hundred and ten examinations it was found one hundred and twenty times, and it is not improbable that by using a larger amount of fluid it might have been found in a great many of the remaining cases.

In frequent instances so enormous must have been the number of organisms in the conjunctival sac, one loopful of fluid produced on the surface of the serum over two hundred colonies of this bacillus in pure culture. It was very common to produce from thirty to one hundred colonies in one inoculation.

To determine the time taken for colonies of xerosis bacillus to become visible to the naked eye, tubes were examined at different periods of incubation. On repeated occasions minute transparent colonies have been observed at the end of sixteen to eighteen hours, which have subsequently been proved to be the bacillus under consideration.

In morphological appearance the xerosis bacillus is very similar to the diphtheria bacillus, but differs from it in cultures. On serum the colonies are small, greyish-white, and very adherent to the surface of the medium; colonies of the diphtheria bacillus in the same time are much larger, whiter, and softer. On agar the xerosis colonies are very small, greyish, and translucent, and on media containing glucose growth is not accompanied by the formation of acid.

No. 2 (table IV) died out before a complete study had been made of its life history; it somewhat resembled a bacillus (diphtheroid I) isolated by EYRE[1] from milk.

No. 3 I have taken to be HOFFMAN's bacillus; it was identical in every respect with a HOFFMAN's bacillus derived from the throat.

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1. *On the presence of members of the diphtheria group of bacilli other than the Klebs-Löffler bacillus in milk. British Medical Journal*, August 18, 1900.

No. 4 only differed from the diphtheria bacillus in its slightly less granular appearance, and in its non-virulence. It is probably an attenuated diphtheria bacillus analagous to that occurring in a throat after an attack of diphtheria, or in the throats of apparently healthy children.

No. 5, although a short variety with few involution forms, produced abundant acid in glucose containing media; it was, moreover, not pathogenic to guinea pigs.

In addition to the five varieties described in the table many intermediate forms have been isolated.

One form with highly segmented protoplasm formed colonies in serum and agar indistinguishable from those of the xerosis bacillus, but in litmus glucose broth at the end of five or six days acid was produced.

Another form, also similar to the xerosis bacillus in cultures, was in microscopical appearance a short oval bacillus closely resembling No. 5; it, however, did not produce acid in glucose containing media.

Other forms differing slightly either in morphology or in cultures from one or other member described in the table have been noticed, and it would appear possible to separate from the eye a complete series of bacilli beginning with the short regular form not producing acid in glucose and non-virulent, and ending with a typical diphtheria bacillus pathogenic to guinea-pigs.

Louis Cobbett[1] recognized five types of diphtheria bacilli occurring in the throat:—

1. Oval bacilli with an unstained septum. Young forms
2. Long, faintly stained, irregularly beaded bacilli
3. Regularly beaded bacilli. Streptococcal forms
4. Segmented bacilli
5. Uniformly stained bacilli

He found acid-producing bacilli identical in appearance both in culture and under the microscope with the diphtheria bacillus which were non-pathogenic.

Whilst the xerosis bacillus occurred one hundred and twenty times, other varieties of the diphtheria group were of comparative rarity in the normal conjunctival sac; in diseased conditions, however, many varieties were often isolated from the same eye, Hoffmann's bacillus occurring with almost as much frequency as the xerosis bacillus. It is worthy of note that the organism (No. 4) most nearly resembling B. diphtheriae was never found in the healthy sac, but was on several occasions found in the discharge from catarrhal ophthalmia and other non-diphtheritic inflammations.

1. *The result of 950 bacteriological examinations for diphtheria bacilli during an outbreak of diphtheria at Cambridge and Chesterton. Journal of Hygiene*, vol. i, No. 2.

TABLE IV

Microscopical Appearances on the different Media	On serum, in 48 hours, a few club-shaped organisms make their appearance, together with segmented bacilli, and metachromatic granules. On agar, in 24 hours there are many segmented forms, and a few club-shaped organisms are seen. On the seventh day the serum cultures do not take the methylene-blue stain, whilst the agar cultures stain well. (The fact that the serum cultures quickly lose their power of taking the stain was noticed also in the case of diphtheria bacillus which had many poorly-stained bacilli even on the third day.) In broth the bacilli occur in clumps; the individuals are shorter than those from serum. On the third day, clubbed and segmented bacilli are seen; also a few bipolarly-stained bacilli.	In 24 hours, on serum, there is a number of large segmented bacilli; a few of the bacilli are slightly clubbed. In seven days, involution forms very numerous; segmented forms abound; there are clubs, segmented ovals, and a few bipolarly-stained bacilli. On agar, in 24 hours, majority of bacilli are long and segmented; there are a few clubs and segmented ovals. Metachromatism present. In broth, the bacilli occur in clumps, and early show segmented forms and clubs.	On serum, on the third day, a few short bacilli are seen with granular staining. On the ninth day, some of the bacilli are seen to have slightly enlarged ends, resembling miniature clubs. On agar, in six days, bipolar staining is seen. On potato, the bacilli are all short and stained at the poles, resembling diplococci. Metachromatism is seen. In broth, a few stain at the poles, but otherwise, after 10 days, there are no irregular forms. In broth, they occur in little clumps. On glycerine-agar, on the fifth day a few short segmented forms are seen.	Involution forms appear on the fourth day in serum cultures as large segmented bacilli; later, peg-top forms appear, and bacilli with polar staining. On agar, involution forms are seen in 24 hours, as segmented club-shaped bacilli. In three days the segmented bacilli have increased in number, and many perfect, club-shaped bodies are seen. Many of the bacilli have become swollen-out in the centre forming spindles. The spindle is a characteristic involution form of this bacillus, and is found in quantity in all cultures. In broth the bacilla occur in small clumps, the individuals being long and slender. In three days, segmented, spindle, and club-shaped bacilli are seen. On potato, in seven days, many and beautiful clubs are formed. On gelatine in seven days no clubs are seen, but many occur that are swollen-out in the centre.	The bacilli from the primary culture adhered so closely together that it was with difficulty that films were spread, but continual sub-culture on agar caused them to lose, to some extent, the property of clumping together in large masses. On the different media the microscopical appearances are fairly constant; the only irregular forms that appear are a few slightly longer bacilli with segmentary staining, having sometimes one end slightly swollen-out into a rounded knob. On potato, there is a very close resemblance to diplococci. After the third or fourth day the bacilli have lost their power of staining with methylene-blue In broth, the bacilli are grouped together in masses of various sizes, which closely resemble collections of cocci. In addition to the short forms are seen slightly longer bacilli with three or four segments. Occasionally one of these longer forms occurred with one extremity slightly swollen. After sub-culture on agar for nearly six months the bacilli show a tendency to become longer and thicker in the centre with the formation of more pronounced involution forms.
Staining Reactions	Stains with all the aniline dyes. Stains by Gram's method.	Stains by Gram's method.	Stains with all the aniline dyes. Stains by Gram's method. Cultures three or four days old stain badly with methylene-blue.	Stains wi h all the aniline dyes. Stains by Gram's method. Cultures on serum on the third day stain badly with methylene-blue, whilst cultures from agar, in marked contrast, stain well even after seven days.	Early cultures stain well with all the aniline dyes, but old cultures do not stain at all with methylene-blue, and only lightly with fuchsin and methyl violet; involution forms stain deeply. Stains by Gram's method.
Motility	None.		None.	None.	None.
Chromogenicity	None.	A light-brownish colour is produced in the serum cultures.	In certain cultures a light-yellow colour develops.	On stab-agar a slight tinge of yellow appeared in the growth.	Cream-yellow colour, which does not infiltrate the medium.
Gas Production	None.	None.	None.	None.	None.
Acid Production	None.	None.	None.	Acid reaction in 24 hours, which increases up to the third and fourth day.	Strong acid reaction in less than 24 hours.
Indol Production	None.		None.	Indol reaction.	Slight indol reaction.
Anaërobiosis	At the end of three days incubation at 38° C, in the absence of oxygen, no growth; subsequently growth in the presence of oxygen.		At the end of three days at 38° C, in the absence of oxygen, no growth. In subsequent incubation no growth occurs, the bacillus having, apparently, been killed.	Does not grow in the absence of oxygen; after three days exposure is still vital.	At the end of three days there is a very slight increase of growth.
Pathogenicity	Not pathogenic.	Not pathogenic.	Not pathogenic.	Not pathogenic.	Not pathogenic.

SUMMARY OF ORGANISMS FOUND IN THE DISEASED CONJUNCTIVAL SAC

The varieties of organisms isolated from inflamed eyes do not differ to any very great extent from the organisms isolated from healthy eyes. In an individual inflamed eye, however, it was very common to find four or five or even more varieties occurring together, whilst from a healthy eye it was very unusual to cultivate more than two or three different kinds of organisms.

After an inflammation of the eye had lasted for a little time, one or other of the pyogenic cocci was commonly found in the discharge, and, as a result of inoculation in a rabbit's eye and in a guinea-pig subcutaneously, it was found to possess considerably more virulence than a similar organism obtained from a healthy sac. There is very little doubt, therefore, that the ordinary pyogenic cocci occurring in chronic diseases of the conjunctiva contribute in some measure to the severity and continuance of the inflammation, and are probably in many cases, particularly in chronic conjunctivitis and in chronic trachoma, the only remaining cause of the continued inflammation.

In illustration of the fact that the pyogenic cocci on the inflamed conjunctiva possess an added virulence, I will mention a case of suppuration of the eye-ball following an operation for cataract. The patient had a little chronic catarrh of the conjunctiva with morning discharge, which was treated with antiseptics for some time before operation was considered advisable. The inflammation of the eye-ball which followed the operation shows how tenaciously suppurative organisms adhere to the hypertrophied conjunctiva, and how extremely difficult it is for antiseptics to act effectually on every corner of a roughened and pitted membrane.

From the purulent discharge in this case cultures of B. xerosis and staphylococcus aureus were obtained. The staphylococcus inoculated on the conjunctiva of a rabbit caused a very intense conjunctivitis.

A fact of aetiological significance is seen in the occurrence of certain organisms in the normal conjunctival sac, which, under suitable conditions, may give rise to an ophthalmia. B. lacunatus (Eyre), streptococcus longus, staphylococcus aureus, and albus, have been met with in the normal conjunctival sac and as causal agents of inflammatory processes. It is not improbable that the Koch-Weeks bacillus can reside in the healthy conjunctival sac without causing inflammation.

ORGANISMS FOUND IN THE DISEASED CONJUNCTIVAL SAC

Gonococcus
Koch-Weeks bacillus
Bacillus diphtheriae
Streptococcus pyogenes longus
Staphylococcus pyogenes aureus

Staphylococcus pyogenes albus
Staphylococcus epidermidis albus
Staphylococcus cereus albus
Staphylococcus cereus flavus
Staphylococcus citreus
Staphylococcus brevis
Pneumococcus (Fraenkel)
Bacillus lacunatus (Eyre)
Bacillus xerosis
Bacillus subtilis
Bacillus capsulatus mucosus
Bacillus coli communis
Bacillus enteriditis (Gartner)
Cladothrix (white)
Penicillium glaucum
Proteus vulgaris
Sarcina lutea
Sarcina aurantiaca
Sarcina alba
Bacillus of Colomiatti. Table III, No. 3
Bacillus striatus flavus
Table III, No. 1
Table III, No. 6
Table IV, No. 4
Table IV, No. 5

Comparative study of the rapidity with which Organisms artificially introduced are removed from the Healthy and Diseased Conjunctivae

Method.—A pure culture of some easily detected organism was inoculated on the conjunctiva. A small quantity of lachrymal fluid was taken after the lapse of varying intervals of time by means of a sterile cotton-wool swab, which was smeared well over the surface of agar.

Experiment 1.—A loopful of sarcina lutea was introduced into a perfectly healthy rabbit's conjunctiva. The organism had entirely disappeared in eighteen hours.

Experiment 2.—Two loopfuls of bacillus coli were introduced into the healthy conjunctival sac of a rabbit. The disappearance was not so rapid as with the sarcina. In twenty-four hours eighteen colonies were grown, and in forty-eight hours, six; at sixty hours the organism was no longer present.

Experiment 3.—Two rabbits were taken whose conjunctivae were in a condition of chronic inflammation.

One loopful of bacillus coli was inoculated into each of the four eyes, and cultures were made on successive days. A growth of the bacillus was obtained up to the ninth day in two cases, and to the tenth and thirteenth in the remaining cases.

In one case, for the first four days, the organism occurred in gradually decreasing numbers, but on the fifth day there was a sudden increase; from this time there was again a decreasing number of colonies obtained, when on the tenth day there was a still further increase. On the thirteenth day and subsequently no colonies were obtained.

In another case there was no increase in the number of bacilli removed until the ninth day. In all the eyes the period during which the inoculated organism could be recovered was prolonged as compared with that observed in the case of the normal eyes.

These experiments show not only that organisms remain for a longer time in the diseased conjunctival sac than in the healthy, but also that organisms are capable of living and multiplying in the folds of the hypertrophied mucous membrane.

Pathogenesis

A number of experiments with the organisms isolated has been performed on animals.

It was not thought necessary to extend the experiments to every one of the common saprophytes of the air and to those bacteria which manifestly could have neither an effect in producing or continuing an inflammation.

Experiments were first performed on the conjunctiva of guinea-pigs, kittens, and rabbits, but the small size of guinea-pigs' conjunctival sacs and the unsuitability of kittens caused these animals to be rejected in favour of rabbits.

The rabbit possesses many advantages over other animals; the upper lid can with the greatest ease be everted and the whole conjunctival membrane exposed; the area of the membrane is considerable; slight friction with a platinum loop seems to cause the animal no inconvenience, and experiments and observations can be made with a minimum amount of movement of the subject.

In addition, the pathogenicity of certain organisms has been tested by inoculation in suitable form in guinea-pigs and mice.

The methods employed have been the same in every instance, and for purposes of comparison the amount of material has been the same in each case.

In the eye experiments the course pursued was as follows:—As early as possible after the primary inoculation the organism was subcultivated on agar or on

serum; a little of this subculture was taken on the loop of a platinum wire and smeared over the whole surface of the rabbit's conjunctiva with gentle friction. Where this rule is departed from mention will be made.

The experiments show that staphylococci from the normal conjunctival sac were considerably less virulent than similar staphylococci occurring in an inflamed conjunctiva. One loopful of a one day old agar culture of any of the staphylococci obtained from a healthy sac produced no reaction in a rabbit's conjunctiva when introduced without injury to the surface of the membrane, whereas simple introduction of staphylococcus aureus, and in one instance of staphylococcus albus, obtained from inflamed eyes was followed by a very appreciable inflammatory reaction.

With gentle friction, however, a very severe reaction followed inoculation of pyogenic staphylococci obtained from inflamed eyes, whilst of the staphylococci obtained from the healthy conjunctiva, staphylococcus aureus was the only one which produced any marked reaction, and this reaction was decidedly less than the inflammation produced by a staphylococcus aureus derived from an inflamed eye.

The following tables show at a glance the methods employed, and the results obtained, in each experiment.

TABLE V. INOCULATION EXPERIMENTS ON THE CONJUNCTIVA. NORMAL FLORA

No.	Organism	Age of Culture	Medium	Amount	With or without friction	Animal	Result
1	Xerosis bacillus	1 day	Serum culture	6 loops	+	Rabbit	No reaction.
2	St. epidermidis albus	1 day	Agar culture	1 loop	+	do.	At the end of 24 hours there was slight reddening of the conjunctiva without discharge. The appearance was normal at the end of 48 hours.
3	St. pyogenes albus	1 day	Agar culture	1 loop	+	do.	*1st day.*—There was a little white pus in the internal canthus and conjunctival sac, and slight congestion of the palpebral conjunctiva. The vessels of the ocular conjunctiva were slightly dilated. *2nd day.*—The inflammation was subsiding, but there was still a little discharge. *4th day.*—The appearance was normal. In one case there was a little undue redness which remained till the fifth day.
4	St. pyogenes aureus (*a*)	1 day	Agar culture	1 loop	+	do.	At the end of the first day there was a slight discharge with redness of the palpebral conjunctiva and slight dilatation of the vessels of the ocular conjunctiva. The membrane was practically normal before the end of four days.
5	(*b*)	1 day	Agar culture	1 loop	+	do.	In 24 hours there was fairly severe reaction, muco-purulent discharge, and redness of the nictitating membrane. The vessels around the cornea and the conjunctival vessels of the upper and lower lid were engorged. There was photophobia. The inflammation decreased after 36 hours and the discharge became less, ceasing at the end of four days. The redness of the palpebral conjunctiva remained for two days longer.
6	St. cereus flavus	1 day	Agar culture	1 loop	+	do.	There was a little discharge in 24 hours, ceasing before 48 hours, and moderate congestion lasting three days.
7	Streptococcus pyogenes longus	1 day	Agar culture	Whole of culture	+	do.	No reaction.
8	Bac. Lacunatus (Eyre)	1 day	Serum culture	Several loopfuls	+	do.	No reaction.
9	Bac. Mucosus Capsulatus	1 day	Agar culture	3 loopfuls	+	do.	No reaction.
10	The same, after passage through guinea-pig	1 day	Agar culture	3 loopfuls	+	do.	No reaction.
11	Bacillus coli communis	1 day	Agar culture	3 loopfuls	+	do.	No reaction.

TABLE VI

Inoculation Experiments on the Cornea

Normal Flora

No.	Organism	Age of culture	Medium	Method	Animal	Result
1	B. xerosis	1 day	Serum	The cornea was abraded with a sterilized needle loaded with a little pure culture	Rabbit	No opacity.
2	St. epidermidis albus	1 day	Agar	do.	do.	No opacity.
3	St. pyogenes aureus	1 day	Agar	do.	do.	Slight cloudiness around the point of inoculation in one day, which extended and increased in density up to the third day, when it began to diminish. On the second day vessels were seen growing out over the cornea from the ocular conjunctiva; these increased in size up to the third day, and formed a beautiful network over the opaque area. At the same time there was some conjunctivitis and discharge, but this cleared up in three days. In a week the opacity had disappeared and the vessels were obliterated.

TABLE VII. Subcutaneous Inoculation Experiments. Normal Flora

No.	Organism	Age of culture	Medium	Amount	Animal	Weight	Result
1	B. xerosis	1 day	Serum	Growth on six tubes emulsified	Guinea-pig	520 grms.	No reaction.
2	St. epidermidis albus	1 day	Broth	3 c.c.	Do.		No abscess formation.
3	St. cereus albus	1 day	Broth	3 c.c	Do.	495 grms.	No abscess formation.
4	St. pyogenes albus	1 day	Broth	3 c.c.	Do.	515 grms.	No abscess formation.
5	St. pyogenes citreus	1 day	Broth	3 c.c.	Do.	422 grms.	In 3 days a small lump was seen which was found to contain a little reddish pus. The organism was cultivated from the pus. Animal recovered.
6	St. pyogenes aureus	1 day	Broth	3 c.c.	Do.	500 grms.	In 1 day oedematous swelling; pus formed on the third day. On the fifth day death with metastatic abscesses. Staphylococcus aureus recovered from the pus.
7	St. pyogenes aureus	1 day	Broth	3 c.c.	Do.	700 grms.	Small indurated lump, which in 4 days did not form into an abscess. On the fourth day the animal was killed; there was no pus found at the point of inoculation.
8	Streptococcus brevis	1 day	Broth	1 c.c.	Mouse		Animal lived.
9	Streptococcus pyogenes longus	1 day	Broth	1 c.c.	Mouse	18 grms.	No reaction.
10	Bacillus mucosus capsulatus	1 day	Agar	Whole of agar culture emulsified	Guinea-pig		At the end of 5 days there was a large, indurated, painful swelling; animal looked ill and had lost weight. In 11 days the swelling had diminished. Animal suffered from diarrhoea. At the end of 15 days the animal was killed. At the point of inoculation there was a tumour the size of a small marble containing a thick white pus. Cultures = pure culture of bacillus mucosus.
11	Bacillus coli communis inoculated into peritoneum	18 hrs.	Broth	3 c.c.	Guinea-pig		Death in 36 hours with general infection. P.-M. Increased amount of fluid in peritoneum; flakes of lymph in the fluid and on the intestines; congestion of mesenteric vessels. The lymph contained bacilli, some of which were in he cells. The blood contained the bacillus. Pure cultures were obtained from the peritoneal fluid and the blood, colonies in the latter case not being numerous.

TABLE VIII. Inoculation Experiments on the Conjunctiva with Organisms from Inflamed Eyes

No.	Organism	Source of organism	Age of culture	Medium	Amount	With or without friction	Animal	Result
1	Xerosis bacillus	Trachoma	1 day	Serum	6 loops	—	Rabbit	No reaction.
2	Staphylococcus epidermidis albus	Do.	1 day	Agar	1 loop	+	Do.	There was a slight reddening of the conjunctiva in 24 hours. The appearance was normal at the end of 48 hours.
3	Staphylococcus pyogenes albus	Dog Ophthalmia	1 day	Agar	1 loop	+	Do.	At the end of 24 hours the eyelids were 'gummed' together; there was great swelling of the upper lid and profuse discharge of white pus: the upper and lower conjunctival membrane was congested and swollen; the vessels of the nictitating membrane were congested, and there was a ring of dilated vessels around the cornea. The cornea was faintly milky all over, bu principally n the upper quadrants. On the second day the oedema was a little less, the discharge remaining about the same in amount. The cornea was distinctly more opaque in the upper quadrants, and a few vessels were seen invading the corneal tissue. On the third day the nflammation commenced to diminish in intensity, and on the seventh day the eye was practically normal. The corneal opacity was the last to disappear.
4	Staphylococcus pyogenes albus	Corneal Ulcer	1 day	Agar	1 loop	+	Do.	In one day there was a fair amount of pus in the conjunctival sac and the internal canthus some dried discharge around the roots of the eyelashes congestion of the palpebral conjunctiva, and slight dilatation of the vessels of the ocular conjunctiva. The cornea was clear. The condition remained the same up to the second day, when the inflammation began to diminish, clearing up before the end of the fifth day.
5	Staphylococcus pyogenes aureus	Chronic Trachoma	1 day	Agar	1 loop	+	Do.	In 24 hours there was a large amount of yellowish-white discharge 'gumming' the lids together; intense injection of conjunctiva and nictitating membrane dilatation of a few of the ocular conjunctival vessels. The upper eyelid was swollen and thickened; the cornea remained clear. On the second day the condition remained practically the same, but there was intense injection of the conjunctiva. Subsequently there was slow improvement, but the conjunctiva never seemed to quite recover its normal condition, the vessels remaining slightly enlarged and tortuous, the conjunctiva thickened and rugose.
6	Staphylococcus pyogenes citreus	Gonorrhoeal Ophthalmia	1 day	Agar	1 loop	+	Do.	At the end of 24 hours there was slight congestion, with a little white secretion in the internal canthus. The conjunctiva was normal in three days.
7	Streptococcus pyogenes longus	Chronic Trachoma	1 day	Agar	Whole of Culture	+	Do.	Beyond a little congestion, there was no reaction.
8	B. lacunatus (Eyre)	Trachoma	1 day	Serum	6 loopfuls	+	Do.	No reaction.

TABLE IX. INOCULATION EXPERIMENTS ON THE CORNEA

No.	Organism	Source	Age of Culture	Medium	Method	Animal	Result
1	Staphylococcus pyogenes albus	Dog Ophthalmia	1 day	Agar	The margins of the cornea at the junction of the two upper quadrants was abraded with a needle loaded with a little pure culture	Rabbit	In addition to the corneal changes there was also a very severe conjunctivitis, with profuse discharge and oedema of the upper and lower lid. At the point of inoculation in one day there was a little point of pus; the cornea in the immediate neighbourhood was quite opaque, whilst the rest of the cornea was cloudy. The opacity increased until on the third day the whole cornea was opaque and vessels had grown for a short distance into the corneal substance. The margins of both upper and lower eyelids presented great irregularity of contour. On the fifth day the rabbit died. P.-M. The cornea was opaque especially in its upper half, vessels having grown for some distance into the corneal substance. There was pus in the anterior chamber, and the lens was swollen and opaque. On the top of the rabbit's skull, beneath the skin, was a small abscess cavity, the pus of which contained Staphylococcus albus, a few colonies of Staphylococcus aureus, and Streptococcus longus.
2	St. pyogenes aureus	Acute Ophthalmia (Koch-Weeks bacillus)	1 day	Agar	do.	Rabbit	In one day, at the point of inoculation, there was a small spot of pus with milkiness of the cornea in the immediate neighbourhood; the milkiness increased in extent and density up to the second day, extending from the point of inoculation to the median line of the cornea. The vessels of the sclerotic above the abrasion were greatly dilated and tortuous, and seemed to send branches into the corneal substance as well as the ocular conjunctival vessels. As a consequence of the presence of the Staphylococcus in the conjunctival sac there was, in addition to the above manifestations, an acute conjunctivitis. At the end of the second day the condition showed slight improvement, and on the fifth day the conjunctivitis had disappeared and the cornea showed no sign of opacity or vascularisation.

TABLE X. Subcutaneous Inoculation Experiments

No.	Organism	Source	Age of Culture	Medium	Amount	Animal	Weight	Result
1	Xerosis bacillus	Membranous conjunctivitis	1 day	Serum	Growth on 3 tubes emulsified in 3 c.c. broth	Guinea-pig		No reaction.
2	Staphylococcus pyogenes albus	Dog ophthalmia	1 day	Broth	3 c.c.	Guinea-pig		Animal died in about 12 hours. Pure cultures obtained from the subcutaneous oedema fluid, but not from the blood or peritoneal fluid.
3	Staphylococcus pyogenes aureus	Muco-purulent Catarrh	1 day	Broth	3 c.c.	Guinea-pig	230 grms.	Animal died in about 24 hours. Pure cultures obtained from the oedema fluid and the peritoneal fluid; a few colonies were cultivated from the blood.
4	ditto	Catarrhal conjunctivitis	1 day	Broth	3 c.c.	Guinea-pig	520 grms.	In 3 days there was a large tense swelling in the region of the inoculation; the swelling increased in size, pointed and burst on the fifth day, leaving a raw surface the size of a shilling. The animal recovered.
5	Streptococcus pyogenes longus	Dacryocystitis	1 day	Broth	1 c.c.	Mouse	18 grms.	Death in 5 days from general infection.
6	Bacillus coli communis (inoculated into peritoneum)	Chronic conjunctivitis	1 day	Broth	3 c.c.	Guinea-pig		Animal died in 36 hours from general infection.

Conclusions

That the normal conjunctival sac contains organisms in a large proportion of cases.

That pyogenic organisms are only occasionally found in the normal sac, and, when they do occur, have to some extent lost their virulence.

That pyogenic organisms obtained from the inflamed conjunctiva are usually considerably more virulent than similar organisms obtained from the healthy conjunctival sac.

PREVIOUS WORK

Only within the last few years has any important work contributed to our knowledge of the bacteriology of the normal conjunctival sac. All investigations have been made to determine the frequency and pathogenicity of the pyogenic cocci and the percentage of sterile sacs.

Morax[11] believed that the normal conjunctival sac was never sterile. He pointed out that pathogenic organisms are rarely found, and in a series of cases examined by him he never found a staphylococcus aureus or a streptococcus.

Fick[5], in forty-nine observations, found the sac sterile six times, and in another series of fifty found the sac sterile thirty-six times. He isolated various bacilli which he had some difficulty in identifying; he also isolated staphylococcus aureus, micrococcus candicans, streptococcus and sarcina lutea.

Gasparrini states that the micrococcus of pneumonia is found in a large proportion of healthy eyes. He injected fresh cultures into the anterior chamber or vitreous of rabbits producing panophthalmitis, and a plastic iritis and atrophy of the eye with older cultures.

Fraenke[6] found the healthy sac sterile in twenty-eight per cent. of his cases, the staphylococcus aureus or albus occurring ten times out of one hundred and fifteen examinations. He was unable to cultivate the xerosis bacillus, whilst Fraenkel and Uhthoff state that it is frequently present in normal eyes.

Widmark[13] brought forward experimental evidence that the pus organisms when introduced into the conjunctival sac of rabbits did not produce catarrhal inflammation. On the other hand, when inoculated into the cornea an intense conjunctivitis resulted, together with keratitis and perforation of the cornea in fifteen per cent. of the cases.

Leber and Weeks obtained no result by inoculating staphylococcus aureus in the human conjunctiva.

Gayet found the staphylococcus aureus in the healthy conjunctival sac.

Gombert,[7] with the bacteria isolated from the conjunctival sac, performed experiments on the rabbit's cornea; three were found to be pathogenic, producing opacity of the cornea; nine were non-pathogenic.

Maerthen[10] described sixteen varieties of cocci, and two of bacilli; the cocci included staphylococcus aureus and albus. Streptococcus was not found.

MacFarland says that the micro-organisms found in the normal sac are of common occurrence in the air. He encountered several bacilli not previously described (Bac. hirsutus, Bac. coerule-faciens, Bac. circumscriptus, Bac. succinatus, Bac. violaceus flavus).

Lachowicz[8] examined sixty-three normal conjunctival sacs, of which sixty-nine per cent. were sterile. He concluded that the micro-organisms present came principally from the air, and that they only stayed there a very short time. In his experiments he showed that pure cultures of streptococcus and xerosis bacilli introduced into the conjunctival sac did not produce the slightest irritation. Staphylococci were also found.

Gifford, in his cases, found exclusively micrococci.

Bach frequently found the pus cocci in healthy eyes. He describes twenty-seven different micro-organisms, eighteen of which were micrococci. He says that in a large percentage of normal sacs bacteria may be demonstrated and that the conjunctiva must be regarded as constantly infected.

Lawson[9] in a series of two hundred cases found the healthy conjunctival sac sterile forty-one times. Serum was used throughout for the primary inoculations. In sixteen cases only were pyogenic cocci isolated; staphylococcus pyogenes albus occurred six times; staphylococcus aureus, once; staphylococcus citreus, twice; staphylococcus cereus albus, four times; Fraenkel's pneumococcus, twice.

Various non-pathogenic organisms occurred, of which staphylococcus epidermidis albus was found fourteen times. Inoculation experiments on the cornea of rabbits and guinea-pigs were without result. Staphylococcus aureus was not tried. He calls attention to the frequency of the so-called xerosis bacillus and the comparative infrequency of pyogenic organisms and their non-virulency. The xerosis bacillus was found in one hundred and eighteen tubes, ninety in pure culture.

Randolph[12] made a series of experiments upon the conjunctivae of one hundred individuals. In thirteen cases the conjunctival sac was sterile, and out of the eighty-seven fertile tubes he observed that eighty-five contained staphylococcus epidermidis albus, whilst only two contained a bacillus. The value of these experiments is somewhat vitiated by the fact that in all the cases agar-agar was used as the culture medium. Xerosis bacillus, Bac. lacunatus (Eyre), and others will only grow readily on serum in primary culture. The experiments are of value in that, although a correct estimate of the bacteria of the conjunctival sac has not been arrived at, they show not only that the sac is rarely sterile but that the organisms generally found therein are of feeble pathogenic power.

Additional experiments were made to prove that the most effectual antiseptic is the conjunctiva itself, and that the use of germicides, such as corrosive sublimate, only handicaps the conjunctival mucous membrane in dealing with pyogenic organisms.

EYRE[3] gives a detailed description of the microscopical and cultural features of the xerosis bacillus which he obtained from cases of follicular catarrh or trachoma. At the end of twenty-four hours on serum no growth was visible to the naked eye or microscopically, but after a period varying from thirty-six to forty-eight hours after inoculation an abundant growth made its appearance. In many of my inoculations growth was visible at the end of sixteen to eighteen hours.

EYRE[4] examined a series of one hundred and fifty healthy sacs from seventy-six individuals, seventy-five of these were sterile. He isolated twenty-eight different varieties of organisms which included most of the pyogenic cocci; staphylococcus aureus occurred sixteen times; citreus, four; albus, thirteen; epidermidis albus, fourteen; cereus flavus, two; and streptococcus longus, three times.

Staphylococcus aureus was inoculated three times into guinea-pigs and was found to be pathogenic.

Staphylococcus citreus in one case possessed considerable virulence, whilst another had but feeble pyogenic powers. Staphylococcus albus was pathogenic to mice in two out of four experiments.

Streptococcus pyogenes longus in each of the three times inoculated in mice caused streptococcic infection, two mice dying in three days, the third in five days.

He pointed out that the alteration which is found to have taken place in the biological characters of organisms obtained from the conjunctival sac is evidence of a very real bactericidal action of the tears, and he further mentions that the rate of growth is appreciably decreased.

Experiments as to the length of time organisms remained in the conjunctival sac were made. A pure culture of B. prodigiosus was introduced into the healthy sac of a rabbit, and at varying intervals 0·05c.c. of lachrymal fluid was drawn off. The number of organisms rapidly diminished, and at three hours 0·05c.c. contained only three colonies, whilst at twenty-four hours no colony could be produced.

Part II

In dealing with the bacteriology of the suppurative inflammations of the conjunctiva I shall not give a complete clinical account of each disease, as most of them are too well-known to need any detailed description. But where necessary for the clear understanding of the exact disease under consideration, a few points in the clinical history will be noted.

A table of all the bacteria found in inflamed eyes has already been drawn up for comparison with those obtained from healthy eyes, and inoculation experiments have shown that the pyogenic cocci from inflamed eyes are, in a large proportion of cases, more virulent than the normal flora.

It is extremely probable that many of the chronic inflammations of the eye, such as cicatricial trachoma and chronic conjunctivitis, are due solely to the continued action of those cocci which have become lodged in the hypertrophied folds of the conjunctiva. In many cases, also, the pyogenic cocci may contribute in some measure to the severity and continuance of an acute imflammatory process.

The material was collected by means of a sterile cotton-wool swab and the platinum loop. In many cases the pus was taken from the upper fornix conjunctivae after the lid had been everted ; by this means a sample of pus was obtained free from any risk of contamination.

The chief medium was again horse-serum, but in many cases the discharge was divided equally over serum, serum-agar, agar-agar, and broth.

In all cases films of the discharge were made. When there was no discharge films were sometimes made of the lachrymal fluid ; the most careful examination of the film in the latter case often failed to show any organism with the exception occasionally of a bacillus presenting granular staining.

Ophthalmia Caused by the Gonococcus

I. Eight cases of gonorrhoeal ophthalmia have been examined. With the exception of one child three years old they were all cases of ophthalmia neonatorum. The gonococcus was observed in large numbers in every instance.

The only point worth calling attention to in these cases was the absence of all other bacteria except the gonococcus in the early stages. Later, numerous and varied organisms could be cultivated. The conjunctiva, in the disorganized state resulting from the inflammation, appears to become a nidus for all bacteria which are deposited on it, and the hypertrophied folds of the mucous membrane provide an excellent soil for their growth and multiplication.

II. In these researches the Koch-Weeks bacillus has been found in four varieties of inflammation about the eye.

(*a*) In acute ophthalmia.

(*b*) In catarrhal ophthalmia.

(*c*) In catarrhal ophthalmia in which the upper lid is covered with small miliary granules (follicular catarrh) and also in a few cases associated with large granules.

(*d*) In mucocele.

Acute Ophthalmia caused by the Koch-Weeks Bacillus

Although the Koch-Weeks bacillus is usually associated with muco-purulent catarrh, yet occasionally it produces a very acute inflammation which cannot clinically be distinguished from gonorrhoeal inflammation.

Four of such cases have come under observation ; two of the cases, before a microscopical examination was made, were diagnosed as gonorrhoeal ophthalmia ; the other two were suspected to be due to infection by the gonococcus.

The ages of the patients were seven months, twelve months, seven years, and thirty-four years, respectively. The last named, a woman with a large family, had had a previous and similar attack a few months before (no history of discharge from the eyes in the family could be obtained).

In the babies, both eyes were almost simultaneously affected ; in the older patients one eye alone was inflamed at first, but after a week or ten days, when the inflamed eye had approached the normal, the disease appeared in the other eye.

Examination of the discharge, after films had been made in the usual way and stained with an aniline dye, showed enormous numbers of a short, slender bacillus.

In all four cases by far the greater number of the bacilli seen in the film was enclosed by leucocytes. Many were scattered about in the fibrin and between the cells, but those inside leucocytes far outnumbered those outside. A few of the leucocytes might be seen containing one or two bacilli only, but usually if leucocytes contained any at all, they were found absolutely packed with them.

It will be seen later that in less acute inflammations caused by the Koch-Weeks bacillus it was sometimes difficult to find a single leucocyte containing the bacillus.

This organism, sometimes, and more correctly, called the bacillus conjunctivitis, is a very slender, short rod closely resembling the bacillus of mouse septicaemia. Its length varies somewhat, but in discharge it rarely exceeds $1{\cdot}5\ \mu$ in length. It is very frequently seen with a constriction in the middle forming two distinct elements, each element having a slightly oval shape ; the dividing line is sometimes difficult to make out. The bacillus never forms chains.

Cultural Peculiarities

The cultivation of the bacillus conjunctivitis was very difficult. It does not grow on the ordinary laboratory media, and only occasionally on coagulated horse serum. Pure cultures, however, have been obtained on horse serum, serum agar, and human blood agar. On the two latter media a few colonies only grew on one occasion, and sub-cultures could not be obtained. On serum there was better success, and sub-cultures were made to the third and fourth generation. The saprophytic growth of the bacillus varies considerably, and seems to depend upon the period it has been exposed to the action of the lachrymal secretion. If cases are taken early, a good growth can be obtained on serum. In the four acute cases a good growth was obtained on serum, and the organism retained its vitality for some time. In muco-purulent catarrh of some duration a good growth was most difficult to obtain, and sub-cultures were not readily made.

During the course of these observations, Reinhard Hoffmann,[23] in a paper on the Koch-Weeks bacillus, mentions that a medium composed of two parts of two per cent. glycerine-peptone agar and one part of human ascites fluid, mixed with sterile wether blood in the proportion of one to two, gave very good results, and that he was able to sub-cultivate the bacillus up to the twenty-fifth generation.

So far as my observations with this medium go, I have been unable to confirm Hoffmann's statements.

On serum the colonies are minute, discrete, transparent, slightly raised growths, with a rounded conical centre and flat smooth margins. The colonies can only be seen with distinctness by the aid of a magnifying lens. In two days they have obtained their greatest magnitude. In one case they were found growing in association with a coccus, and in several cases they occurred in mixed culture with the xerosis bacillus.

Microscopically, the colonies consist of very slender, non-motile rods, varying in length but not in thickness ; some of them grow out into fairly long curved threads. Many of the shorter bacilli are divided by a barely perceptible division. Most of the bacilli are cylindrical, but a few show slight irregularities of contour, which may be evidence of commencing subdivision. No chains or degeneration forms are seen.

They stain well with fuchsin, but lightly with methylene blue and dahlia ; the stain is not retained by Gram's method. They are non-motile.

Inoculation experiments on rabbits with pure cultures were unsuccessful, but with the mixed growth of Koch-Weeks bacillus and coccus a slight catarrh was produced, lasting for three days, and manifesting itself chiefly in the discharge of a small quantity of white muco-pus. Microscopically, the pus contained numbers of Koch-Weeks bacillus.

The interest of these cases lies in the fact that they may readily be mistaken for infection by the gonococcus. Clinically, in the case of the two children it was

impossible to differentiate between the two diseases; they offered the clinical picture of a gonorrhoeal ophthalmia with the exception that the cornea did not seem to be endangered.

In the older patients the age, the character of the discharge, the previous attack, the absence of corneal complications and gonorrhoeal history would lead one to suspect that gonorrhoeal ophthalmia was not being dealt with. With regard to the character of the discharge, in the two latter cases the discharge, although profuse, was of a whitish colour and stringy consistence, and was with difficulty removed from the conjunctival sac; gonorrhoeal pus is invariably yellow and friable.

The previous attack is a very important aid to diagnosis; it lends colour to the supposition of Reinhard Hoffmann[23] that the bacillus conjunctivitis is able to exist and lie dormant for a long time in the slightly hypertrophied folds of the conjunctiva resulting from a former attack, and become a means of propagation of the disease to other people and a danger to the patient in recurring attacks.

The woman must, in all probability, have been harbouring the bacillus in the recesses of her conjunctiva during the period between the two attacks, and, owing to lowered general vitality or diminished local resistance, the bacillus was able to again manifest its presence. In her first attack the disease remained confined to one eye, but in the second the other eye became involved ten days afterwards.

This form of ophthalmia differs from the classical muco-purulent catarrh in the severity of the symptoms; it is characterized by intense pain, photophobia, and redness of the conjunctiva, profuse discharge and often large sub-conjunctival ecchymoses.

In the case of the woman, after the inflammation had subsided, the upper lid was everted, and the conjunctiva examined. The membrane was very congested, and presented a roughened appearance; the roughness did not amount to actual miliary granulations.

III. Muco-purulent Catarrh caused by the Koch-Weeks Bacillus

The Koch-Weeks bacillus varies considerably in virulence. In the disease now under consideration, the invasion of the conjunctiva by the organism causes only a mild type of inflammation, and, in comparison with the acute ophthalmia described above, frequently causes only slight congestion of the palpebral conjunctiva.

The discharge contains a large proportion of fibrin and occasionally forms a pseudo-membrane.

Microscopical films of the discharge show a large amount of fibrin enclosing polynuclear leucocytes, epithelial cells, and bacteria.

In recent cases the slender bacilli are very numerous, and if the inflammation be fairly acute many leucocytes are seen containing bacilli. The bacilli were often seen adhering to epithelial cells.

As the disease diminishes in severity the number of leucocytes containing bacilli, and the number of bacilli in the discharge, correspondingly diminish.

At first no other organisms could be observed in microsopical preparations, and in cultures from very early cases, besides the Koch-Weeks bacillus and a few colonies of the xerosis bacillus, no other organisms could be cultivated.

As the disease became more advanced the adventitious organisms increased in number and variety; the pyogenic cocci, and especially the staphylococcus aureus, became frequent inhabitants of the sac.

As convalescence approached the enormous increase in the numbers of the xerosis bacillus was very remarkable. In the small amount of discharge at this period the bacillus was sometimes so abundant that films appeared to be made from a pure culture.

Previous Work on the Subject of Infection by Koch-Weeks Bacillus

The bacillus was first seen by Koch[28] (1883) in the discharge from cases of acute catarrhal ophthalmia. He examined in Egypt fifty cases of ophthalmia in which he found two microbes; the one, Neisser's gonococcus, associated with severe symptoms; the other, a small slender bacillus associated with mild symptoms. He ascribed the propagation of the disease to flies which were often seen covering the faces of children. Cultural experiments were unsuccessful.

In 1887 Kartulis described the bacillus in Egyptian ophthalmia and corroborated Koch's observations. He describes the bacillus in pure culture and succeeded in producing the disease in man in one out of six inoculation experiments. The description of his cultures would apply to the cultural appearances of the xerosis bacillus, but it is not unlikely that, since the slender bacillus may sometimes be found growing in association with or in the neighbourhood of other organisms, in his successful inoculation the culture contained Koch-Weeks bacillus.

In 1887 Weeks[45] published a memoir in New York on the results of his investigations on catarrhal ophthalmia. He found in all his cases a small slender bacillus but was unable to obtain pure cultures, a club-shaped organism being always associated with it. Inoculation of the discharge on animals gave a negative result, but he was able to reproduce the disease in man by employing the mixed cultures.

In 1895 Weeks[46] in a further communication stated that, in over a thousand cases of catarrhal ophthalmia examined by him, Koch-Weeks bacillus was a constant factor in producing the disease.

Gromakowski[18] in eighteen cases of acute conjunctivitis found a slender bacillus which he concluded had an etiological relationship with an acute, highly epidemic conjunctivitis.

MORAX[31] concluded that the bacillus was constantly present in muco-purulent catarrh. He described pure cultures of the bacillus and stated that inoculation of these cultures on his own conjunctiva produced acute inflammation.

He also further describes the histological appearances of a piece of his conjunctiva which was snipped off. The bacilli in sections of this tissue were with difficulty demonstrated in the superficial layers of the conjunctiva ; in the deeper layers they could not be detected.

Later, JULER mentioned that he had met the organism in many, but not in all, cases of acute ophthalmia.

MORAX and BEACH[33] published a full account of their experiments with the bacillus which they cultivated on agar-agar with or without blood serum.

WILBRAND, SAENGER, and STAELIN[44] describe an epidemic of conjunctivitis in Hamburg in which two varieties of micro-organism were found, one of which was Koch-Weeks bacillus.

JULER, PANAS, and COPPEZ[4] succeeded in observing the bacillus. COPPEZ draws attention to the often pseudo-membranous character of the inflammation to which I have already alluded.

GASPARRINI[15] mentions the occurrence of the bacillus in Italy.

SIDNEY STEPHENSON[40 & 41] gives a description of the outbreak and course of a small epidemic in the Central London District School, in which the characteristic small bacilli were found. He states that the presence of Koch-Weeks bacillus in the discharge is diagnostic of acute catarrhal ophthalmia, and is of the same opinion as CUENOD,[5] that the club-shaped organism described by other writers on the subject is pseudo-diphtheritic.

He found the bacillus in three types of acute inflammation—

1. Classical catarrhal ophthalmia.
2. A form associated with large phlyctenulae in and about the conjunctiva.
3. A variety in which follicular enlargement is superadded, and also in a child suffering from chronic dacryocystitis.

He succeeded in growing the organism on serum-agar, but states that after three to four days it dies, showing degeneration forms.

WEICHSELBAUM and MULLER[47] (1898) published a very comprehensive work on the Koch-Weeks bacillus. They succeeded in isolating the bacillus in pure culture and establishing its etiological significance by successful inoculations on the human conjunctiva.

Within the last year Dr. REINHARD HOFFMANN[23] published an account of his cultural and inoculation experiments with this bacillus. He succeeded in growing it on swine serum-agar and 0·5 per cent. hydrocele fluid agar, but was able to subcultivate it only up to the fifth generation. He remarks that he obtained a growth on agar where a large amount of discharge was lying. The medium which gave him

the best results and enabled him to subcultivate the organism up to the twenty-fifth generation was a mixture of one part of the blood of a wether with two parts of a two per cent. glycerine-peptone agar and human ascites fluid in the proportion of one to two. Even in the oldest cultures he was not able to detect degeneration forms. Inoculation experiments on animals with the discharge and pure cultures were without result. Successful inoculation was carried out on himself and two of his colleagues, an inflammation resulting lasting over a week with the characteristic bacilli present. He concludes that Koch-Weeks bacillus is the cause of an acute, often croupous, very contagious eye inflammation in man, which may become chronic and result in a papillary hypertrophy of the conjunctiva.

Also that in the folds of the conjunctiva the bacillus can remain for a long time and be a possible source of transmission to other individuals and a danger to the individual himself in a re-awakening of the inflammation.

IV. Acute Ophthalmia in which B. Diphtheriae and Streptococcus Pyogenes were Associated

A case of acute ophthalmia was recently obtained which occurred in a female child one year old. One eye alone was affected. The inflammation was very intense, the discharge profuse, and the upper lid greatly thickened. Films of the discharge showed cocci, mainly in pairs, but also occurring in short chains ; a few leucocytes contained cocci in chains of four and singly.

In one instance a leucocyte was seen containing a bacillus stained deeply at the poles. A few of these bacilli were also seen scattered about between the cells.

Cultures of staphylococcus aureus, streptococcus pyogenes longus, and a bacillus closely resembling B. diphtheriae were obtained.

A one day old serum culture of the bacillus was emulsified in one c.c. of broth and inoculated subcutaneously into a guinea-pig, 485 grammes in weight. The guinea-pig died in two and a half days. At the point of inoculation there was a greyish-white purulent exudate with a little surrounding oedema. Pure cultures of the bacillus were obtained from the pus at the point of inoculation.

V. Acute Ophthalmia in a Cat caused by Streptococcus Pyogenes Longus

The cat had, twenty-four hours before the observations were made, been bitten by a rat, the teeth of which had caused a penetrating wound of the right lower conjunctival membrane.

When seen, both eyelids were greatly swollen and oedematous ; the edges of the lids adhered, and on separation a thick white pus exuded ; the ocular and palpebral

conjunctivae were red and oedematous, large subconjunctival ecchymoses having taken place between the sclerotic and the ocular conjunctiva ; the cornea was bright and remained unaffected throughout. The acute symptoms quickly subsided without treatment, recovery taking place in four to five days.

Films of the discharge were made, streptococci in chains being the only organism observed.

VI. Ophthalmia caused by Staphylococci

Two cases of inflammation of the eye have been examined in which microscopically and culturally only the staphylococci could be observed.

Sydney Stephenson[42] records a case of acute ophthalmia, associated with pustular eruptions on the face and scalp, in which staphylococcus aureus and albus alone occurred. In my cases staphylococcus aureus and albus were found associated together. No other organism occurred to which the inflammatory condition could be ascribed.

The inoculation experiments with the organisms obtained from healthy eyes demonstrate that the staphylococci are able to cause an acute inflammation, and it is not improbable that in certain conditions of lowered general or local vitality the staphylococci which happen to be present in the conjunctival sac during such conditions are capable of originating an acute inflammation.

It is undoubted that in some chronic inflammatory conditions the continued inflammation is chiefly due to the presence of the organisms of suppuration.

VII. Acute Ophthalmia in a dog caused by Staphylococcus Albus

The dog had had an attack of inflammation some weeks previously which had incompletely resolved.

This first attack followed directly on the formation of a discharging sore on the upper eyelid through the biting of flies ; the inflammation was not intense, and subsided in about three weeks, leaving the conjunctival mucous membrane slightly hypertrophied. After a month had elapsed the eye again became acutely inflamed. The eyelids were puffy and gummed together, enclosing a large amount of yellow pus. The conjunctiva was very red, swollen, and rough. One eye alone was affected at first.

In a short time after the onset of the inflammation the cornea was noticed to be becoming hazy ; the haziness was general and increased rapidly in density, until the whole cornea was densely opaque and pearly-white. The inflammation soon extended to the other eye, which exhibited the same symptoms in a modified degree.

After several weeks the inflammation became much less intense, and the opacity less, the cornea at last becoming perfectly clear and, apparently, in no way injured.

Examination of the discharge showed only cocci, many of which were in a state of sub-division. The cocci stained by GRAM's method.

Repeated cultural experiments were made, and in every case a number of opaque white colonies of a very virulent coccus was obtained.

On serum the colonies grew rapidly and caused a slight depression on the surface.

On agar an abundant opaque-white spreading growth was produced.

Milk was rapidly coagulated, the reaction becoming strongly acid.

Gelatine showed a large amount of liquefaction in twenty hours.

Culturally the organism resembled staphylococcus pyogenes albus, only producing equal results with greater rapidity.

The first attack was not investigated, but it is very improbable that the staphylococcus originated the disease.

The more likely assumption is that the disorganized conjunctiva was infected by the staphylococcus secondarily.

According to FLUGGE,[9] staphylococcus pyogenes albus is more common than aureus among many of the lower animals.

VIII. Granular Ophthalmia

The causal agent of trachoma has for a long time been involved in obscurity, and a series of cases has been examined to see if some light could be thrown on the subject.

SATTLER (1885) isolated a micrococcus from the trachomatous follicles in cases of Egyptian ophthalmia, and MICHEL (1886) who gave a more exact description of the coccus, made inoculation experiments which he believed established the etiological relationship to the form of ophthalmia with which it was associated. These researches have not been confirmed, and subsequent observers have not been able in many cases to find the micrococcus.

KARTULIS, FUCHS, and HOOR[11] held the theory that trachoma is often of gonorrhoeal origin. DEMETRIADES[6] considered Egyptian ophthalmia to be a combination of trachomatous disease with purulent ophthalmia as the gonococcus was always found in the discharge. In no instance in the series of cases described below was the trachomcoccus cultivated, or the gonococcus observed in the discharge.

It was considered imperative, in order to find the exciting cause of trachoma, to obtain the cases in the early stage. This presented some difficulty, in that granules are generally of slow formation, and it was not possible to tell whether an inflammation

or a catarrh would eventually terminate in trachoma. But, owing to the opportunities of observing children with eye inflammations in the Workhouse Infirmary, it has been noticed that many children admitted with simple muco-purulent catarrh have returned at some period after their discharge suffering from granular lids.

In some instances, all the members of a large family have been inmates of the infirmary at the same time, some suffering from muco-purulent catarrh, others from granular lids.

Old cases of trachoma, although possessing some bacteriological interest, are useless for the purpose of ascertaining the primary cause of the condition, inasmuch as the organism initiating the disease has probably long since disappeared.

The bacteriology of granular conjunctivitis has been described in three sub-divisions :—

(*a*) Early cases showing very small granules scattered over the whole surface of the upper conjunctiva.

(*b*) Later cases with large granules.

(*c*) Old chronic cases with no characteristic granules but a large amount of cicatricial contraction.

In the first series of cases there may or may not be discharge ; the discharge is usually not abundant, and many most pronounced granular lids have only a little discharge 'gumming' the lids in the morning.

In a few instances the small granules, when treatment has been neglected, have been observed to increase in size and merge into the second group.

In addition to the large granules the upper lid often shows a certain amount of new formation of fibrous tissue, causing slight thickening of the lid and alteration in the contour of the palpebral fissure.

The last stage of the disease is marked by the total disappearance of the granules, distortion of the upper lid with dense fibrous tissue, contraction of the conjunctival sac, pannus and other corneal complications.

Probably all cases of trachoma have commenced with the formation of small granules. The majority of adults with classical trachomatous lids state that they have had the complaint 'ever since childhood.'

The disease is often so insidious in its onset, and its symptoms so slight, that a whole school may be affected without any suspicion of its presence until a few children have discharge from the eyes.

As a result of this insidious onset many cases are not seen until the disease is well advanced and the granules large. It is sometimes difficult to obtain a history long enough to account for the presence of large granules. They occasionally grow remarkably quickly and form mushroom-like elevations on the conjunctiva similar to the warty growths of gonorrhoea in the urethra.

TABLE I

Catarrhal Ophthalmia with Small Granules on the Upper Conjunctiva (Follicular Conjunctivitis)

Case	Age	Sex	Amount of Discharge	Organisms Isolated
1	2½ years	Male	Scanty	Koch-Weeks bacillus. Xerosis bacillus.
2	5½ years	Male	Scanty	Koch-Weeks bacillus. Xerosis bacillus.
3	6 weeks	Female	Very scanty	Koch-Weeks bacillus. Xerosis bacillus. Staphylococcus epidermidis albus.
4	5 years	Male	Small	Koch-Weeks bacillus. Xerosis bacillus. Staphylococcus epidermidis albus.
5	3 years	Female	None	One or two slender bacilli seen. Xerosis bacillus. Streptococcus brevis. Staphylococcus epidermidis albus.
6	3 years	Female	Small	Koch-Weeks bacillus. Xerosis bacillus. Pseudo-diphtheria bacillus.
7	21 mths.	Female	Small	Koch-Weeks and xerosis bacillus. No. 1, Table III (Part I).
8	21 mths.	Male	Small	Koch-Weeks bacillus. No. 1, Table III (Part I).
9	5½ years	Female	Large	Koch-Weeks bacillus and xerosis bacillus.
10	13 years	Female	Small	Koch-Weeks and xerosis bacillus. Streptococcus longus. Sarcina lutea. Pseudo-diphtheria bacillus.
11	24 years	Female	Small	Koch-Weeks and xerosis bacillus.
12	5 mths.	Male	Small	Koch-Weeks and xerosis bacillus.
13	3½ years	Male	Small	Koch-Weeks bacillus and xerosis bacillus
14	5 years	Female	Scanty	Koch-Weeks and xerosis bacillus. Staphylococcus pyogenes albus.
15	3 years	Female	Small	Bacillus lacunatus (Eyre). Staphylococcus pyogenes albus. Xerosis bacillus (in enormous numbers). One or two slender bacilli.
16	Child	Female	Small	Pneumococcus. Streptococcus brevis.
17	11 years	Female	Scanty	Xerosis bacillus. Staphylococcus pyogenes aureus and citreus.
18	9 years	Female	None	Bacillus capsulatus mucosus. Bacillus coli communis.
19	3 years	Male	None	Xerosis bacillus. Staphylococcus citreus.
20	2 years	Female	None	Koch-Weeks bacillus. No. 3, Table IV. Xerosis bacillus.

In catarrhal ophthalmia with some small granulations on the upper lid, Koch-Weeks bacillus is almost a constant factor in the discharge.

A glance at the table will show that out of a series of twenty observations Koch-Weeks bacillus occurred in fourteen in the discharge, and was doubtful in a fifteenth.

The first five cases are from a series of six which were left for a month without treatment, to see if residence in the country with plenty of fresh air and good food had any influence in causing the disappearance of the small miliary granules.

It was then found that instead of having diminished in size, the granules had undergone a very appreciable enlargement, and the whole of the upper palpebral

conjunctiva was studded with pale pinkish elevations. The discharge in each case was not abundant, and when present appeared as one or two small pellicles in the upper and lower fornix conjunctivae.

In the fifth case, where there was no discharge, one or two slender bacilli were seen in films of the lachrymal fluid.

The sixth case of this series had large granules, and has been placed in the second sub-division.

Cases 15 and 16 were complicated by the presence of B. lacunatus (EYRE) and the pneumo-coccus, and the inflammation was probably due to their presence. In one of them, one or two slender bacilli were detected in the discharge.

In 17, 18, and 19, there was practically no discharge, and films were not made. The four last cases had been for about a week under treatment, and the inflammatory process had been to some extent arrested, yet in the last case careful examination of a little discharge showed one or two slender bacilli.

The organism that occurred most frequently was the xerosis bacillus. This bacillus is found so often in healthy and diseased conditions that it must be considered a regular inhabitant of the conjunctival sac.

Its repeated occurrence in all forms of granular lids made it necessary to prove that it could have no action in causing the granular condition.

With this object in view various experiments were performed on the conjunctiva of rabbits.

1. A pure culture was emulsified in sterile broth, and about 0·25 c.c. inoculated beneath the conjunctiva. On the next day the fluid was absorbed, and subsequently, with the exception of a little redness about the point of inoculation, there was no inflammatory manifestation.
2. Together with two loopfuls of the pure culture a little finely-powdered glass was rubbed gently over the conjunctiva: slightly momentary irritation ensued, which quickly subsided. In twenty-four hours the appearance was normal and there was no congestion.
3. Every day for fourteen days one loopful of a one day old serum culture of the xerosis bacillus was gently smeared over the conjunctiva. On the next day after each inoculation the conjunctiva was normal, and at the end of fourteen days or subsequently there was no appearance of granules.

Additional evidence against the probability of xerosis bacillus being in any way responsible for granular lids is gathered from its occurrence in all diseased states of the conjunctiva. In xerosis of the conjunctiva, in which it was first described by KUSCHBERT and NEISSER, it is present in enormous numbers, and one small loopful of lachrymal fluid from the healthy conjunctiva has been found to contain over two hundred bacilli.

An interesting fact observed many times, is that, with approaching convalescence from inflammatory states, this bacillus is found sometimes in the discharge in very large numbers.

A striking instance of this was seen in an infant recovering from muco-purulent catarrh, whose mother (case 11) suffered from granular lids. When first examined the discharge consisted mainly of fibrin, and contained very few organisms. Seven days afterwards the small amount of whitish discharge which was obtained practically consisted of xerosis bacilli.

In one case of granular lids a typical appearance of xerosis of the conjunctiva was noticed.

The part the bacillus plays in the several processes of the eye has yet to be determined, but the observation is well-founded that the diminution of inflammation seems to be the signal for the growth of the xerosis bacillus.

The other organisms found in catarrhal ophthalmia with small granules were not constant in their incidence, and occurred in the healthy eye and in other inflammatory conditions.

Table II

Granules Large (True Trachoma)

No.	Age	Sex	Amount of Discharge	Organisms
1	2½ years	Female	Large	Koch-Weeks bacillus. Staphylococcus albus and aureus
2	...	Female	Large	Koch-Weeks bacillus
3	13 years	Male	None	One or two slender bacilli. B. xerosis
4	...	Female	Small	One or two slender bacilli. B. xerosis. Staphylococcus pyogenes albus
5	2 years	Male	Scanty	B. xerosis. Staphylococcus epidermidis albus. No. 3, Table IV (Part I)
6	21 mths.	Male	Small	B. lacunatus (Eyre). Staphylococcus pyogenes albus and citreus. No. 3, Table IV
7	7 years	Male	Large	B. lacunatus (Eyre). B. xerosis. Staphylococcus pyogenes aureus and albus
8	34 years	Female	Small	Pseudo-diphtheria bacillus (No. 5, Table IV)
9	16 years	Female	Scanty	B. xerosis. Staphylococcus pyogenes albus
10	12 years	Female	Scanty	B. xerosis. Sarcina lutea. Streptococcus brevis
11	21 years	Male	None	B. xerosis (enormous numbers)
12	2½ years	Male	Scanty	B. xerosis. Staphylococcus epidermidis albus

In the first two cases, where the discharge was considerable, Koch-Weeks bacillus was observed in quantity.

In three and four, where there was no discharge, one or two slender bacilli were observed after examining a large field. It was only possible to say that these slender bacilli resembled exactly the morphological appearance of Koch-Weeks bacillus ; in cases where the bacillus was with difficulty found no cultures could be obtained.

Case five belongs to the series of six which was kept for a month under observation. The discharge in this case was almost wholly fibrin, only a few leucocytes and epithelial cells being seen ; in films of the discharge no organism could be detected.

Case eight was examined four times at different periods in six months, and on every occasion a pseudo-diphtheria bacillus was obtained in pure culture; its characters are given in Table IV (No. 5). This was the only occasion on which this variety of pseudo-diphtheria bacillus was isolated.

A glance at Table II (page 135) shows that no organism occurs with sufficient constancy to justify a causal connection with granular lids.

Also each organism isolated was inoculated on the conjunctiva of a rabbit and in no case did a granular condition result.

TABLE III

Chronic Trachoma with Cicatricial Thickening

No.	Age	Sex	Condition of Lids	Organisms
1	15 years	Female	Some large granules with cicatricial thickening of upper lid	Staphylococcus aureus. Xerosis bacillus.
2	Adult	Female	Entropion. Opacity of cornea	B. lacunatus (Eyre) and xerosis. Staphylococcus aureus and epidermidis albus.
3	Adult	Female	Pannus. Large granulations and thickening of upper lid	B. xerosis. Staphylococcus epidermidis albus.
4	Adult	Female	Ectropion. Conjunctiva red, velvety, and thickened	Streptococcus longus. Staphylococcus citreus. B. xerosis.
5	17 years	Male	Thickening and distortion of upper lid. Opacities of cornea. Obliteration of upper and lower fornices with strong fibrous bands	Fraenkel's pneumococcus. B. lacunatus and xerosis. Staphylococcus aureus and citreus.
6	25 years	Male	Upper lid thickened. Conjunctival surface smooth and whitish. Adherence of ocular and palpebral conjunctiva, causing contraction of the conjunctival sac	Xerosis bacillus.
7	20 years	Male	Ectropion. Conjunctiva hypertrophied, red, and velvety	Staphylococcus aureus. Xerosis bacillus.
8	Adult	Male	Conjunctiva velvety and thickened. Old opacities of cornea	Xerosis bacillus.
9	Adult	Female	Cicatricial distortion. A few large granules	Streptococcus longus. Xerosis bacillus.
10	Adult	Female	Upper lid very thickened. Conjunctival surface smooth and whitish	Xerosis bacillus.
11	26 years	Male	Upper lid thickened. Conjunctiva hypertrophied, red, and velvety	Xerosis bacillus. Staphylococcus aureus.
12	14 years	Male	Entropion. Fibrous adhesions between ocular and palpebral conjunctivae. Some large granules	B. lacunatus (Eyre). Staphylococcus pyogenes albus and citreus Sarcina lutea. Xerosis bacillus.
13	Adult	Female	Entropion. Conjunctiva covered with large irregular granules. Fibrous adhesions between the ocular and palpebral conjunctiva. Extreme pannus	Streptococcus pyogenes longus. Staphylococcus epidermidis albus.

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With scarcely one exception the disease in Table III was of considerable duration; most of the patients could give no definite history, with the exception that they had had 'sore eyes' since childhood. It would, therefore, not be expected that in such a series uniformity in the incidence of a specific organism would be secured, and in the consideration of the agents causing trachoma this table has not been taken into account.

The pyogenic cocci were of frequent occurrence; staphylococcus aureus was found five times; staphylococcus citreus, three times; staphylococcus albus, once; streptococcus longus, three times; pneumococcus, once.

B. lacunatus (EYRE) was isolated on three occasions, once associated with FRAENKEL's pneumococcus. On each occasion there was considerable inflammatory reaction, and it is probable that the inflammation caused by the bacillus was superadded to the granular lids. B. lacunatus rarely causes an acute conjunctivitis, but the conditions for its growth are much more favourable in a conjunctiva disorganized by granules than in a previously normal one. Xerosis bacillus occurred in every case, in some of them in large quantity.

In two of the cases, 6 and 10, where all sign of granules had disappeared and the conjunctiva had undergone natural recovery, xerosis bacillus was obtained in pure culture. Staphylococcus aureus was almost invariably found when the conjunctiva was thickened and velvety.

The almost constant appearance of Koch-Weeks bacillus in the first group, makes the conclusion inevitable that this bacillus is very often the cause of an inflammation of the conjunctiva, accompanied by the formation of granules. It has been seen that when these cases have been left untreated for some time the granules grow in diameter. Also, not only have cases been observed to develop granular lids from simple muco-purulent catarrh, but also several families have come under observation, in which the individuals have each suffered from muco-purulent catarrh, or some form of granular lids, the cause of the muco-purulent catarrh being in all cases Koch-Weeks bacillus.

These facts are strong evidence that the different varieties of granular lids are stages of the same disease, and that in all probability all cases of trachoma have commenced in the formation of the minute miliary granules, as a result of infection by Koch-Weeks bacillus.

The view is now generally accepted that trachoma is a specifically contagious disease due to some organism. In epidemics of trachoma in schools, muco-purulent catarrh often accompanies and equals trachoma in incidence.

The failure to find Koch-Weeks bacillus in more than four cases (? two) of the second group may be accounted for by the fact that—

(*a*) Several had lasted over a year.
(*b*) Discharge was scanty or even absent.
(*c*) Treatment had been given in many cases.
(*d*) The disease was complicated by infection by B. lacunatus (EYRE) in two cases.

Moreover, when the discharge is very scanty, one or two isolated slender organisms cannot be definitely stated to be Koch-Weeks bacillus, and attempts to obtain cultures under these circumstances are usually unsuccessful. It is also extremely probable that, a granular condition having been set up by the Koch-Weeks bacillus, slight forms of irritation, microbial or otherwise, which would have little effect on the normal conjunctiva, are sufficient not only to cause a continuance of the inflammation, but also to produce an actual increase in the size of the granules.

Additional support to the view that Koch-Weeks bacillus is the causal agent in trachoma, is afforded by—

(*a*) The association of muco-purulent catarrh and granular lids in epidemics of ophthalmia in Egypt.
(*b*) The observations of many writers, that inflammations of the conjunctiva caused by the Koch-Weeks bacillus often leave behind a granular condition.

HISTOLOGY OF THE TRACHOMA GRANULE

In a former paper contributed to the Thompson Yates Laboratory Reports, vol. ii, 1900, I have described the histological characters of trachomatous lids.

The granules were found to be chronic inflammatory nodules, consisting of small round cells and a delicate reticulum of fibrous tissue enclosed by an incomplete capsule of bands of fibrous tissue.

Examples of the different stages in the formation of a granule were obtained. Sections of an acutely inflamed conjunctiva showed a general infiltration of the tissue with small round cells and here and there small circumscribed masses of cells which did not possess a capsule. In sections of an inflamed conjunctiva in which there were small granules, the round cell masses were more distinctly circumscribed and, although not possessing a capsule, had a well-defined reticulum of fibrous tissue. A later development is the formation of a fibrous capsule and evident signs of cicatrization of the nodule. In all the cases plasma cells were abundant, and several appeared to have ruptured and discharged their granules into the surrounding tissue.

With regard to the presence of organisms in the sections, no very positive result was obtained, but in one or two instances bodies that had a great resemblance to the slender bacilli were observed; these were especially noted in a cover-glass preparation of a crushed follicle.

Previous Literature

Shongolowicz[39] considers the importance of examining the tissues as well as the discharge for organisms. In the crushed contents of follicles he found very small short rods which stained badly and chiefly at the poles. They grew on all media, but poorly ; they grew best on flesh peptone-agar, producing a greenish colour.

Inoculation of rabbits and cats produced a certain similarity to trachoma in man.

Shongolowicz does not doubt that he has found the true cause of trachoma, and ascribes the discovery of cocci by Sattler to the difficulty of staining the organism, and the fact that it stains bipolarly. (It is not unlikely that this organism staining bipolarly was the xerosis bacillus).

In twenty-six cases of trachoma he found staphylococcus albus in twelve ; staphylococcus citreus in three ; staphylococcus aureus in nine ; staphylococcus cereus albus in three ; and in seven cases the short bacillus.

Muller,[34] from the secretion in trachoma disease, obtained a slender bacillus, which resembled morphologically and culturally the influenza bacillus. In fifteen cases he found the bacillus eleven times. He further remarks that he found the bacillus in old cicatricial trachoma only when there was a certain amount of discharge.

The description of the bacillus would apply to the Koch-Weeks bacillus, and in this respect his observations agree with mine.

Logetschnikow[30] thinks that follicular catarrah and trachoma are not one and the same, their identity has yet to be proven. He thinks that the micro-coccus of Michel should be more appropriately designated as the coccus of follicular catarrh instead of trachom-coccus.

Schmidt,[38] in forty-seven out of fifty-eight cases, failed to obtain cultures of the trachom-coccus, which he found to possess great morphological resemblance to the staphylococcus pyogenes. Inoculation produced a severe muco-purulent conjunctivitis in dogs, rats, and rabbits. In pigeons, he states that the cocci produced typical trachoma.

Sternberg[43] remarks that the description of the trachom-coccus would apply to some of the more common pus cocci, *e.g.* Staphylococcus pyogenes aureus, which have also been shown to consist of two hemispherical halves separated by a narrow line of sub-division.

Kartulis failed to find Sattler's trachom-coccus or any other organism in the contents of a trachomatous follicle. He states that if treatment be neglected in muco-purulent catarrh caused by Koch-Weeks bacillus, a granular infiltration of the conjunctiva results, which subsequently offers the clinical picture of trachoma.

Wilbrand, Saenger, and Staelin[44] observed that in conjunctivitis caused by Koch-Weeks bacillus changes remained behind which suggested true trachoma, but they add that in these cases follicle formation has existed from the beginning.

HOFFMANN[23] states that cases which have hitherto been taken for papillary trachoma are cases of conjunctivitis caused by Koch-Weeks bacillus which have become chronic.

REICH[37] calls attention to the fact that although follicles of not specifically trachomatous character may be observed, we are unable to distinguish them from the milder forms of trachoma.

FULTON[11] regards follicular conjunctivitis and trachoma as pathologically the same disease and believes in the direct contagiousness of both.

In COPPEZ'[4] opinion follicular and granular conjunctivitis are identical.

ZIEGLER[48] observes that follicular catarrh and trachoma are indistinguishable, but that follicular catarrh never quite produces the cicatricial degeneration seen in true trachoma. He further states that follicular catarrh can be produced by atropine and that it is simply the expression of a chronic irritation ; also that it seems to be originated by a diplococcus resembling the gonococcus, but which stains by GRAM.

LEBER[29] describes the presence of certain large cells containing peculiarly formed bodies in trachoma, and he thinks that these have probably to do with the pathogenesis of the affection.

GERMANN[12] in three cases of acute trachoma states that the infection had apparently been brought about by black earth in the conjunctival sac. He considers that the distribution of trachoma is effected by the dust of the fields and agricultural employments in which much dust is engendered.

HIRSCH[20] and HIRSCHBERG[21] give some points on the distribution of trachoma in certain districts of Europe. TRUC suggests methods for the prevention of the distribution of the affection. In their works no bacteriological or histological examinations were made.

HERBERT[19] states that in trachoma and conjunctivitis the formation of adenoid tissue from connective tissue can be traced, and that in follicular and granular conjunctivitis the new formations might differ from the normal in being deficient in supporting stroma and blood vessels.

Professor GUARNIERI[17] describes some bodies, staining intensely with magenta red, one-third to one-half the size of red blood corpuscles, which he obtained after grattage of trachomatous lids. He suspects that they are of a parasitic nature and belong to the blastomycetes ; he did not succeed in cultivating them.

PICK[36] states that—

(*a*) The follicles are not immediately contiguous to the epithelium, but are separated from it by bands of fibrous tissue circularly arranged around the nodule.

(*b*) The follicle cells are of the same kind as the round cells found in the conjunctival stroma.

(*c*) The trachoma bodies of Burchardt are found only in the epithelium and have absolutely nothing to do with protozoa.

STEPHENSON[41] states that muco-purulent catarrh does not last long, and even when not subjected to treatment does not produce any serious or permanent lesion of the eye. He says that the most important forms of disease which are prevalent in schools are follicular ophthalmia and trachoma. The supposed transition of follicular disease into trachoma has arisen, it is thought, from children with follicular disease being placed in wards with trachomatous children from whom they have contracted that infectious disease. Very few instances of trachoma occurred in children under two years of age.

IX. Diplo-Bacillary Conjunctivitis

In the out-patient department of the Royal Infirmary a number of patients applying for treatment for refractive errors was met with, who, on enquiry, gave a history of a little discharge in the morning 'gumming' the lids and sensations of burning or grit in the eyes.

No definite history as to duration could be obtained ; they had all suffered from morning discharge for some time. Twelve cases have been investigated ; the patients were all middle-aged women and at the time of observation the disease was symmetrical.

When the lids were everted the conjunctiva was seen to be congested and slightly velvety, but even in the most protracted case there was no cicatricial distortion or thickening of the eyelids.

In some of the cases there was a small pellicle of white muco-pus floating in the lachrymal fluid, and perhaps a little dried secretion around the roots of the eyelashes.

In films of the discharge the organism most frequently observed was a short thick bacillus, commonly seen in pairs, but also in short chains of eight or more elements.

Cultures on Serum. After twenty-fours incubation at 38° C. the surface of the serum was eroded with numerous shallow pits which, on further incubation, grew rapidly in depth and extent, liquefying the serum.

At first no growth was visible, but later a little greyish growth appeared at the bottom of the pits.

Film preparations of this growth showed a short thick bacillus ; involution forms were early and exceedingly irregular in shape.

In nine out of twelve cases this bacillus was cultivated.

Analysis of Twelve Cases

Case		Organisms Isolated
1	...	B. lacunatus (EYRE).
2	...	B. lacunatus.

Case		Organisms Isolated
3	...	B. lacunatus. Staphylococcus aureus. B. xerosis. Pseudo-diphtheria bacillus.
4	...	B. lacunatus.
5	...	B. lacunatus.
6	...	B. lacunatus.
7	...	B. lacunatus. Staphylococcus epidermidis albus and aureus. B. xerosis.
8	...	B. lacunatus. Staphylococcus aureus.
9	...	B. lacunatus. B. xerosis.
10	...	Staphylococcus albus and epidermidis albus. B. xerosis.
11	...	B. xerosis.
12	...	B. xerosis. Staphylococcus albus.

The bacillus has been met with frequently in children, but always complicating granular lids ; it was isolated nine times from conjunctivae apparently healthy. On a few occasions a bacillus closely resembling it has been observed in blepharitis in the discharge around the eyelashes.

BIARD[3] states that the diplo-bacillus is never present in the normal conjunctival sac, but is a constant inhabitant of the nasal fossa, and he concludes that infection takes place either by direct extension or by transference by means of the fingers.

MORAX[32] and AXENFELD[2] simultaneously and independently described a diplo-bacillus which they isolated from subacute cases of conjunctivitis. AXENFELD also found it in acute cases. EYRE[8] found the bacillus in many cases of conjunctivitis in which the chronicity of the inflammation and the contagious nature of the discharge were marked. He states that both sexes, and all ages, are susceptible, but that it is most common in middle-aged women. The objective signs were slight, being confined to a little mucus-like discharge adhering to the roots of the eyelashes and collecting in the neighbourhood of the caruncle, also slight injection of the bulbar conjunctiva, and marked injection of the palpebral conjunctiva with an erythematous fringe along the free edge of the lower lid.

X. AFFECTIONS OF THE LACHRYMAL SAC

Only five cases of swelling of the lachrymal sac have been met with. Two were cases of mucocele in which the contents were clear and viscid ; three were cases of acute purulent dacryocystitis.

In brief, the results of the bacteriological examination were as follows :—

MUCOCELE

Case	
1	Koch-Weeks bacillus. Staphylococcus epidermidis albus.
2	Koch-Weeks bacillus. Staphylococcus pyogenes albus. Pseudo-diphtheria bacillus.

Acute Dacryocystitis

Case		
1	Girl, aged 13.	Streptococcus pyogenes longus.
2	Girl, aged 10.	Streptococcus pyogenes. B. xerosis and pseudo-diphtheria bacillus. Staphylococcus citreus. White cladothrix.
3	Girl	Streptococcus pyogenes.

The streptococcus from case 1 was inoculated in a mouse, which died in five days from general infection.

These observations confirm the results of Morax, Parinaud[35], and Eyre[7].

Morax found Koch-Weeks bacillus in a large number of his cases of mucocele.

Morax and Parinaud isolated streptococcus pyogenes from the pus of acute inflammation of the lachrymal sac. Eyre investigated twenty-six sac cases. In ten cases of acute purulent dacryocystitis streptococcus was constant, and in a large proportion of the chronic cases streptococcus occurred. In six cases of mucocele Weeks bacillus was isolated three times.

Unclassified Cases

In all about two hundred individuals with inflamed eyes have been examined; in many, discharge has been collected from both eyes. A large number of the cases has not been classified; they include, mainly, simple inflammatory states that have been for some time under treatment, chronic inflammatory states with no definite history, traumatic cases, etc.

A few cases of blepharitis and ulcer of the cornea have been examined, but the number has not been sufficient for any definite conclusions to be arrived at. In blepharitis staphylococcus aureus frequently occurred, and in some cases a short bacillus was obtained, which closely resembled the diplo-bacillus of Morax.

In one case of ulcer, with bulging of the cornea, a little of the discharge removed from the surface of the cornea was found to contain remarkably few organisms of any kind: cultures of B. lacunatus (Eyre); staphylococcus epidermidis albus; B. xerosis and a pseudo-diphtheritic bacillus were obtained.

In a case of membranous conjunctivitis, closely resembling the conjunctivitis produced by B. diphtheriae, only B. xerosis and a pseudo-diphtheritic bacillus were isolated. Inoculation of a large quantity of these bacilli into guinea-pigs produced no reaction.

A pure case of pneumococcus ophthalmia was not met with; on the one occasion on which it occurred it was complicating cicatricial trachoma, and was associated with B. lacunatus (EYRE). Inoculation of a pure culture into a mouse showed that it had lost its virulence.

Two cases of epithelial xerosis of the conjunctiva, one associated with night blindness and no evident disease of the palpebral conjunctiva, the other associated with granular lids, were investigated. The white flakes removed from the conjunctiva practically consisted of the xerosis bacillus.

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BILE SALT BROTH

BILE SALT BROTH

I. A SIMPLE TEST FOR FAECAL CONTAMINATION

II. THE BEHAVIOUR IN BILE SALT BROTH, IN CERTAIN SUGARS, AND IN GLYCERINE, OF SOME OF THE COMMONER ORGANISMS—WITH SPECIAL REFERENCE TO THE EFFECT OF THEIR PRESENCE UPON THE VALUE OF THE ABOVE TEST

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I

In a previous number of these reports (*T. Y. Reports*, vol. iii, part 2) one of us (MacConkey) described a medium which gives a most characteristic reaction when inoculated with *B. coli communis* or *B. typhi abdominalis.* This is an agar medium containing taurocholate of sodium and lactose. Upon this the colonies of B.C.C. grow freely and rapidly ; they assume a yellow colour and give rise to a fine precipitate in their vicinity—thus producing the appearance of a halo—on the other hand the colonies of B.T.A., though not usually apparent at the end of twenty-four hours, are after forty-eight hours plainly visible as whitish translucent roundish colonies without any surrounding haze. Further it was found that the medium was unfavourable to the growth of all the commoner forms of spore-bearing organisms—such as *B. subtilis* and its allies—which are usually present in water, whilst the temperature of incubation 112·42°C. further inhibited the growth of most of the ordinary water bacteria.

In order to simplify this test further—by the use of a fluid instead of a solid medium—a taurocholate glucose broth has been devised.

The composition of the medium is as follows : —

Sodium Taurocholate	0·5 per cent.
Glucose	0·5 ,,
Peptone	2·0 ,,
Water	100 cc.

These constituents are heated together until the solids are dissolved and then filtered. After filtration, sufficient neutral litmus solution to give a distinct colour is added. The medium is then distributed into test tubes, and one of DURHAM's fermentation tubes placed in each. Sterilization is effected in twenty minutes by KOCH's sterilizer on each of three successive days. After the third sterilization the small inner tube should be quite full. Glucose was used instead of lactose in order not to exclude the B. enteritidis of GAERTNER and allied organisms, as they are incapable of fermenting lactose.

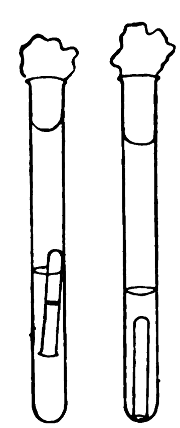
The percentage of glucose was determined by careful experiments, which led to the conclusion that any higher percentage was detrimental to the growth of the B.C.C. and allied organisms.

The absence of sodium chloride and the extractives of meat enables the medium to be of an almost uniform composition, of which the advantage is obvious. The temperature of incubation is 42° C., the same as that for the agar medium ; this enables the reaction to occur quickly and favours the growth of all intestinal organisms. The litmus indicates at once whether there is acid formation. The DURHAM's tube shows whether there is fermentation of the glucose.

The taurocholate favours the growth of the group of organisms which are the most important from the point of view of faecal contamination, viz., the B.C.C. and GAERTNER groups, etc.

CHARACTERISTIC REACTION

On inoculating this medium with B.C.C. or the GAERTNER bacillus, the following characteristic reaction is produced within forty-eight hours. The appended drawings show it clearly.



The medium is uniformly red in colour. The fermentation tube is filled with gas, and the surface of the medium is covered over with a mesh work of bubbles, whilst a constant stream of bubbles can be seen rising to the surface. After inoculation with B.T.A., the medium becomes uniformly red in colour, but there is no production of gas as the bacillus is incapable of fermenting glucose.

Time of incubation

In order to make the test as rapid as possible, it was, at the outset, necessary to determine some limit for the time of incubation. This we have fixed at forty-eight hours, as we are satisfied, from numerous experiments, that if B.C.C., etc., be present, the reaction occurs within this limit. In the majority of cases, it is within twenty-four hours.

Precautions to be observed when using the test

1. *Formation of Gas.* Frequently a few small bubbles collect in the upper part of the fermentation tube after incubation. This is apparently due to evaporation of liquid from the tube owing to the high temperature, and can readily be recognized. It is totally different in appearance to the fermentation caused by the reacting organisms, which is general: the whole liquid being permeated with rising bubbles of gas, and the surface covered with a meshwork of the same.

2. *Pipettes.* It is advisable to use a separate pipette for making each dilution, and for inoculating the tubes; but if this be impracticable, one pipette should be used for making the dilution, and a second for inoculating the tubes, care being taken to commence the inoculation at the highest dilution.

3. *Plates.* When it is desired to make plate cultivations from the Taurocholate broth, this should be done forty-eight hours after inoculation, as the development of acid tends to kill off the organisms.

We have examined the behaviour in this medium of ninety-four organisms, most of which were obtained from Král's laboratory.

Grouping of organisms

Four headings obviously present themselves under which the organisms can be grouped :—

I. Those producing acid and gas.
II. Those producing acid only and no gas.
III. Those capable of growth in the medium, but producing neither acid nor gas.
IV. Those incapable of growth in the medium.

and finally a fifth group :

V. Those incapable of growth on any medium at the temperature of incubation, viz., 42° C.

Division I contains seventeen organisms :—

1. B. coli communis (Escherich) (subculture of original strain)
2. B. acidi lactici (Hueppe)
3. B. cavicida (Brieger)

4. B. coli communis (Laboratory stock culture)
5. B. neapolitanus
6. B. capsulatus (PFEIFFER)
7. B. lactis aerogenes (ESCHERICH) (subculture of original strain)
8. B. enteritidis (GAERTNER)
9. B. icteroides (SANARELLI) (subculture of original strain)
10. B. paracolon (LE SAGE)
11. B. of epidemic jaundice
12. B. psittacosis (NOCARD)
13. B. of hog cholera (SALMON and SMITH)
14. B. pneumoniae (FRIEDLÄNDER)
15. B. der frettchenseuche (EBERTH)
16. B. cloacae (JORDAN)
17. M. candicans (FLÜGGE)

Division II contains twenty-two organisms :—

18. B. typhosus
19. B. pyogenes foetidus
20. B. of dysentery (KRUSE)
21. „ (FLEXNER)
22. „ (SHIGA)
23. „ (GRAY)
24. „ (HARRIS)
25. Vibrio cholerae (BERLIN)
26. „ „ (MARSEILLES)
27. „ „ (ELVERS)
28. „ „ (FROHNER)
29. „ „ (ARGEL)
30. „ Metschnikovi
31. B. rhinoscleroma
32. Proteus vulgaris
33. B. prodigiosus
34. B. pseudo-tuberculosis (PFEIFFER)
35. B. der Darm-Diphtherie (RIBBERT)
36. B. fluorescens liquefaciens (FLÜGGE)
37. Staphylococcus pyogenes aureus
38. „ „ albus
39. „ „ citreus

Division III contains fifteen organisms:—

40. B. butyricus (Hueppe)
41. B. butyricus
42. B. filamentosus
43. B. buccalis maximus
44. B. mesentericus vulgatus
45. B. mesentericus fuscus
46. B. mesentericus niger
47. B. pyocyaneus
48. B. Zopfii
49. B. disciformans (Zopf)
50. B. brunneus
51. B. megatherium
52. V. Finkler-Prior
53. M. ureae
54. Sarcina lutea

Division IV contains nineteen organisms:—

55. B. anthracis
56. B. anthracoides
57. B. diphtheriae
58. B. faecalis alkaligenes
59. B. fluorescens albus
60. B. fluorescens putidus
61. B. necro-dentalis
62. B. Stutzeri
63. B. subtilis
64. B. xerosis
65. M. tetragenus
66. Sarcina aurantiaca (Flügge)
67. Sarcina flava (De Bary)
68. Sarcina ventriculi
69. Saccharomyces ellipsoideus
70. Cladothrix dichotoma
71. Torula alba
72. Torula rubra
73. Streptococcus pyogenes

Division V contains twenty-one organisms:—

74. B. arborescens
75. B. aureus
76. B. candicans

77. B. diffusus
78. B. fluorescens aureus
79. B. fluorescens mesentericus
80. B. fuscus
81. B. gelber (KORN)
82. M. mycoides
83. B. proteus mirabilis
84. B. proteus Zenkeri
85. B. ramosus
86. B. of haemorrhagic septicaemia
87. M. agilis
88. Oidium lactis
89. Spirillum rubrum
90. Saccharomyces cerevisiae I
91. Saccharomyces urinae
92. B. pestis
93. St. cereus albus
94. B. Violaceus

On examining the list of organisms contained in groups I and II, it will be seen that the large majority are intestinal in origin. Those of group I, viz., those producing the reaction of gas and acid formation, are with one or two exceptions obviously intestinal organisms. *It is, therefore, justifiable to conclude, that when the reaction is obtained, it is most probably produced by organisms of intestinal origin.*

When an incomplete reaction is obtained, viz., acid formation only and no gas, there is still strong suspicion of intestinal pollution, as it will be seen most of the organisms in group II (including Typhoid, Dysentery, and Cholera) are of the same origin. *Conversely it may be stated, that when the reaction is not present faecal contamination is absent.*

Practical application of the test

To prove the practicability of this test, samples of drinking and river water sent to the Laboratory have been tested by this method. Similarly, various dilutions of crude sewage and sewage effluents have been subjected to this fermentation test. Comparative tests with the taurocholate agar have in many instances been made side by side.

Method

In the case of drinking water, 1 cc. of the water is added to the broth in each of three separate tubes respectively, the three tubes are incubated at 42° C. for forty-eight hours, and the results read off.

In the case of crude sewage, effluents, or river water, definite dilutions of the cubic centimetre are made in each instance, viz., $\frac{1}{10}$ $\frac{1}{100}$ $\frac{1}{1,000}$ $\frac{1}{10,000}$ $\frac{1}{100,000}$, according as it is desired to determine whether there are 10, 100, 1,000, 10,000, 100,000 B. coli present in the cubic centimetre of the sample (other dilutions can also be made as occasion demands). Four distinct dilutions are severally tested, and three determinations made of each dilution. Thus, twelve tubes of the medium are used for each sample. The tubes are incubated at 42° C. for forty-eight hours, as in the previous instance.

It is found in practice that in the large majority of cases the characteristic reaction is obtained within twenty-four hours, frequently within eighteen hours. In the case of the higher dilutions more time seems to be requisite, so that it is advisable to incubate for forty-eight hours. If the reaction has not occurred within that time, it can be definitely stated that further incubation will not produce it. The medium may become acid or slowly decolourized, but the formation of gas is never observed; when it occurs, it takes place rapidly.

Results

A. *Drinking water—*

1. *Moorland filtered water:*

Samples	118
Number of reactions . . .	5
„ „ due to B.C.C. .	5

2. *River filtered water:*

Samples	169
Number of reactions . . .	16
„ „ due to B.C.C. .	16

B. *River water:*

Samples	41
Number of reactions . . .	41
„ „ due to B.C.C. .	41

C. *Milk:*

Samples	103
Number of reactions . . .	98
„ „ due to B.C.C. .	98

D. *Sewage and sewage effluents:*

The reaction has always been obtained in every sample examined.

Conclusions

Above it has been stated that, considering the origin of the organisms that gave this reaction, it was justifiable to conclude that when reaction was obtained, faecal contamination was almost certainly present. The results obtained with waters and milk tend to confirm this conclusion.

We think, therefore, we are warranted in putting forward this test as a rapid means of detecting faecal contamination.

DIVISION I

Organism	Taurocholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	Glycerine 48 hrs.	Glycerine 6 days
1 B. coli communis (Escherich)	A and G	A and G	A and G	A and G	nil	A and C	Opaque-white, many with orange centre. Haze	White, non-liquefying	D	A
2 B. acidi lactici (Hueppe) (K)	A and G	A and G	A and G	A and G	nil	A and C	Opaque-white. Haze in depths	White, non-liquefying	D	A and G
3 B. cavicida (Brieger) (K)	A and G	A and G	A and G	A and G	nil	A and C	Round, flat, opaque white, some with orange centre. Haze	White, non-liquefying	D	A and D
4 B. coli communis	A and G	A and G	A and G	A and G	A and G	A and C	Opaque-white, many with orange centre. Haze	White, non-liquefying	D	D
5 B. neapolitanus	A and G	A and G	A and G	A and G	A and G	A and C	Like B.C.C. (Escherich)	White, non-liquefying	D	A and D
6 B. capsulatus (Pfeiffer) (K)	A and G	A and G	A and G	A and G	A and G	A and C	*Surface:* translucent, white, with clear ring around. *Deep:* orange, with haze	White, non-liquefying	A and G	
7 B. lactis aerogenes (Escherich)	A and G	A and G	A and G	A and G	A and G	A and C	Like B.C.C. (Escherich)	White, non-liquefying	A and G	...
8 B. enteritidis (Gaertner)	A and G	A and G	D	A and G	D	A	Flat, translucent, greyish white. No haze	White, non-liquefying	D	A and D
9 B. Icteroides (Sanarelli)	A and G	A and G	D	A and G	D	slight A	Like B. enteritidis	White, non-liquefying	D	A, very slight
10 B. Para Colon (Le Sage)	A and G	A and G	D	A and G	D	slight A	Like B. enteritidis	White, non-liquefying	D	A, very slight
11 B. of epidemic jaundice	A and G	A and G	D	A and G	D	slight A	Like B. enteritidis	White, non-liquefying	D	A, very slight
12 B. psittacosis (Nocard)	A and G	A and G	D	A and G	D	A	Like B. enteritidis, but with filmy edge. No haze	White, non-liquefying	D	D
13 B. of hog cholera (Salmon & Smith)	A and G	A and G	D	A and G	D	A	Like B. enteritidis, but with flat top and bevelled edge. No haze	White, non-liquefying	D	A and D
14 B. pneumoniae Friedländer)	A and G	A and G	nil (later A)	A and G	A and G	A	Round, raised, opaque white. No haze	White, non-liquefying	Nch	Nch
15 B. der Frettchenseuche (Eberth) (K)	A and G	A and G	A and G	A and G	A	A and C	Round, raised, opaque, yellowish white. Haze	White, non-liquefying	D	A and G
16 B. cloacae (Jordan) (K)	A and G	A and G	A	A and G	A and G	A (later C)	Opaque-white, mucoid. No haze	White, non-liquefying	Nch	A, very slight
17 M. candicans (Flügge) (K)	A and G	A and G	A	A and G	A and G	A (later C)	Opaque-white, mucoid. No haze	White, non-liquefying	Nch	A

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

* Nos. 6, 7, and 14 ferment starch.

DIVISION II

Organism	Tauro-cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum.	Glycerine 48 hrs.	Glycerine 6 days
18 B. typhosus	A	A	D	A	nil	nil or slight A	*Surface:* semi-transparent, greyish-white. *Deep:* opaque-white	Grey, non-liquefying	nil	A
19 B. pyogenes foetidus (K)	A	A	A	A	A	A and C	Like B.C.C. (Escherich)	White, non-liquefying	nil	A and D
20 B. dysentericus (Kruse)	A	A	nil	nil	nil	nil	Flat, opalescent, very like B. enteritidis	White, non-liquefying	nil	A, very slight
21 B. dysentericus (Flexner)	A	A	nil	nil	nil	nil	„ „	White, non-liquefying	nil	A, very slight
22 B. dysentericus (Shiga)	A	A	nil	nil	nil	nil	„ „	White, non-liquefying	nil	A, very slight
23 B. dysentericus (Gray)	A	A	nil	A	nil	A	„ „	White, non-liquefying	nil	A, very slight
24 B. dysentericus (Harris)	A	A	nil	nil	nil	A	„ „	White, non-liquefying	nil	A, very slight
25 V. cholerae (Berlin) (K)	A	A	A	A	A	A	Flat, transparent, greyish-white. Slight haze	Pink, liquefying	nil	A
26 V. cholerae (Marseilles) (K)	A	A	A	A	A	A	„ „	Pink, liquefying	nil	A
27 V. cholerae (Elvers) (K)	A	A	A	A	A	A	„ „	Pink, liquefying	nil	A
28 V. cholerae (Frohner) (K)	A	A	A	A	A	A	No growth	Pink, liquefying	nil	A
29 V. cholerae (Argel)	A	A	A	A	A	A	No growth	Pink, liquefying	nil	A
30 V. Metchnikovi (K)	A	A	nil	A	A	slight A	No growth	Pink, slight-liquefaction	nil	A
31 B. Rhinoscleroma	A	A	nil	nil	nil	A and C	*Surface:* Flat, greyish-white, translucent. *Deep:* like balls of wool, appearing hollow from below. No haze	Scanty	nil	A
32 Proteus vulgaris (K)	A	A	D	nil	nil	slight A	Round, raised, translucent, greyish-white. No haze	Yellowish-white, non-liquefying	nil	A
33 B. prodigiosus	A	A	nil	A	A	A	No growth	Scanty	nil	nil
34 B. pseudo-tuberculosis (Pfeiffer) (K)	A	A	nil	nil	nil	nil	No growth	Greyish-white, non-liquefying	nil	nil
35 B. der Darm-Diphtherie (Ribbert) (K)	A	A	nil	nil	nil	nil	No growth	White, non-liquefying	nil	nil
36 B. fluorescens liquefaciens (Flügge) (K)	A	A	nil	nil	slight A	A and C	No growth	Pink, slow-liquefaction	slight A	A
37 St. pyogenes aureus	A	A	A	nil	A	A and C	*No growth	Yellow, non-liquefying	nil	A
38 St. pyogenes albus	A	A	A	nil	A	A and C	*No growth	White, non-liquefying	nil	A, very slight
39 St. pyogenes citreus	A	A	nil	nil	A	slight A	*No growth	Yellow, non-liquefying	nil	A, very slight

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization (K) Obtained from Král's laboratory.

* These organisms seem capable of growth on this medium, when freshly isolated.

DIVISION III

	Organism	Taurocholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	Glycerine 48 hrs.	Glycerine 6 days
40	B. butyricus (Hueppe) (K)	Growth	A	A, very slight	slight A	slight A	slight A C P	No growth	Pinkish white, liquefying	nil	nil
41	B. butyricus	Growth	A	nil	nil	nil	A C P	No growth	White, non-liquefying	nil	nil
42	B. filamentosus (K)	Growth	A	nil	nil	nil	A C P	No growth	Pink, liquefying	nil	A
43	B. buccalis maximus	Growth	A	nil	nil	nil	A and C	No growth	White, slow liquefaction	nil	A
44	B. mesentericus vulgatus (K)	Growth	D	nil	A, very slight	D	slight A C and P	No growth	Rapid liquefaction	nil	A
45	B. mesentericus fuscus (K)	Growth	D	nil	A, very slight	D	slight A C and P	No growth	Slow liquefaction	nil	A
46	B. mesentericus niger (K)	Growth	D	nil	A, very slight	D	slight A C and P	No growth	Coarse feathery outgrowths, liquefying	nil nil	A
47	B. pyocyaneus	Growth	nil	nil	nil	nil	A C P	Flat, transparent, pinkish brown. No haze	Pink, rapid liquefaction, complete colouration of serum	nil	Green
48	B. Zopfii	Growth	nil	nil	nil	D	nil	*Surface:* grey, translucent, with filmy, hairy edge. No haze. *Deep:* like balls of wool	White, feathery, non-liquefying	nil	A
49	B. disciformans (Zopf) [(K)	Growth	slight A	nil	nil	A	A C P	No Growth	White, slow liquefaction	nil	A
50	B. brunneus	Growth	nil	nil	nil	nil	Alkaline	Round, raised, opaque-greyish white. No haze	White, non-liquefying	nil	A
51	B. megatherium	Growth	nil	nil	nil	nil	A and C P	No growth	Slight liquefaction	nil	A
52	V. Finkler-Prior	Growth	nil	nil	nil	nil	slight A	No growth	Pink, liquefying	nil	nil
53	M. ureae (K)	Growth	nil	nil	nil	A	A, very slight	No growth	White, non-liquefying	nil	nil
54	Sarcina lutea	Growth	nil	nil	nil	nil	nil	No growth	Yellow, liquefying	nil	A, very slight

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

DIVISION IV

Organism	Tauro-cholate Glucose	Glucose	Lactose	Mannite	Cane	Litmus Milk	Colonies on Taurocholate Lactose Agar	Blood Serum	Glycerine 48 hrs.	Glycerine 6 days
55 B. anthracis	no growth	A	nil	nil	A	nil	No growth	Greyish, non-liquefying	nil	A
56 B. anthracoides (K)	no growth	D	nil	D	slight A	A C P	No growth	Abundant, white, non-liquefying	nil	slight A
57 B. diphtheriae	no growth	nil	nil	nil	nil	nil	No growth	White, non-liquefying	nil	nil
58 B. faecalis alkaligenes (K)	no growth	nil	nil	nil	nil	nil	No growth	Slow growth, non-liquefying	nil	nil
59 B. fluorescens albus (K)	no growth	D	nil	A, very slight	D	slight A C and P	No growth	No growth	nil	A
60 B. fluorescens putidus	no growth	nil	nil	nil	nil	nil	No growth	White, slow-liquefaction	nil	nil
61 B. necrodentalis [(K)	no growth	nil	nil	nil	nil	A	No growth	No growth	nil	nil
62 B. Stutzeri (K)	no growth	nil	nil	nil	nil	A	No growth	Pinkish, non-liquefying	nil	nil
63 B. subtilis	no growth	A	nil	nil	A	A C P	No growth	Pink, liquefying	nil	A
64 B. xerosis	no growth	nil	nil	nil	nil	nil	No growth	...	...	...
65 M. tetragenus	no growth	nil	nil	nil	nil	nil	No growth	Opaque-white, non-liquefying	nil	nil
66 Sarcina aurantiaca (Flügge) (K)	no growth	nil	nil	nil	nil	nil	No growth	Orange, non-liquefying (later liquefaction)	nil	nil
67 Sarcina flava (De Bary) (K)	no growth	nil	nil	nil	nil	nil	No growth	Yellow, liquefying	nil	nil
68 Sarcina ventriculi (K)	no growth	nil	nil	nil	nil	nil	No growth	Brown, non-liquefying (later liquefaction	nil	A, very slight
69 Saccharomyces ellipsoideus	no growth	nil	nil	nil	A	nil	No growth	Scanty, white, non-liquefying	nil	nil
70 Cladothrix dichotoma	no growth	nil	nil	nil	nil	nil	No growth	Brown, liquefying	nil	nil
71 Torula alba	no growth	nil	nil	nil	nil	nil	No growth	...	...	...
72 Torula rubra	no growth	nil	nil	nil	nil	A	No growth	No growth	...	...
73 Streptococcus pyogenes	no growth	...	A	...	...	A, very slight	No growth	...	...	...

A. Acid. G. Gas. C. Clot. D. Decolorization. Nch. No Change. P. Peptonization. (K) Obtained from Král's laboratory.

II

In the first part of this paper we have shown how it is possible to group the chief micro-organisms with regard to their behaviour and growth in Taurocholate-glucose broth.

In order to further separate and distinguish the various members of each group, we have studied their fermentative power upon the following sugars, viz., glucose, lactose, mannite, and cane, upon glycerine, their reaction in litmus milk, and their growth on blood serum and Taurocholate-lactose agar. The results are shown below in tabular form.

Temperature and length of time of incubation

In this table in every instance the temperature of incubation has been 42° C. The duration of incubation has been forty-eight hours, except where otherwise stated.

Nature of media employed

The composition of the various media employed is as follows:—

Litmus taurocholate glucose broth: Sod. taurocholate, 0·5 per cent.; glucose, 0·5 per cent.; peptone, 2·0 per cent.; water, 100 cc.

Litmus glucose broth: Glucose, 0·5 per cent.; peptone, 2·0 per cent.; water, 100 cc.

Litmus lactose broth: Lactose, 1·0 per cent.; peptone, 2·0 per cent.; water, 100 cc.

Litmus mannite broth: Mannite, 1·0 per cent.; peptone, 2·0 per cent.; water, 100 cc.

Litmus cane sugar broth: Cane sugar, 1·0 per cent.; peptone, 2·0 per cent; water, 100 cc.

Litmus glycerine broth: Glycerine, 1·0 per cent.; peptone, 2·0 per cent; water, 100 cc.

Taurocholate lactose agar: Agar, 1.5 per cent.; sod. taurocholate, 0·5 per cent.; lactose, 1·0 per cent.; peptone, 2·0 per cent.; water, 100 cc.

An examination of the table of reactions of Division I leads to the conclusion that certain organisms, which have been described as distinct from each other, are in reality very closely allied, or identical. Thus, Nos. 1, 2, and 3 are practically alike. It has been said that No. 2, B. acidi lactici (HUEPPE), is stained by GRAM's method. It is true that a faint coloration may be observed after this method of staining, but we consider that this amount of colour does not warrant the statement that it 'stains by GRAM.' We think that the result of GRAM's method should be considered positive only when the organism is of the well known blue-black colour.

Even though Nos. 4, 5, 6, and 7 differ from the preceding in their fermentative action on cane sugar, we do not think that this justifies separation into a special group. No. 15 must also be put in the same category.

No. 16 seems to be closely allied to the above organisms, but slowly liquefies gelatine.

The same remarks apply to No. 17. Throughout the whole of our numerous experiments we have never yet met with this micro-coccus; therefore, though it is said to occur in the air, it is improbable that it will invalidate our proposed test for faecal contamination. As regards No. 14, the culture with which we have experimented differs from the usual description of this organism, in that it does not ferment lactose or glycerine. The usual description would lead to its inclusion in the coli group.

The remaining organisms, Nos. 8 to 13, may be classed together, as forming the 'GAERTNER' or 'para-colon' group.

Passing on to Division II, No. 18, B. typhi abdominalis appears to stand out by itself.

No. 19, B. pyogenes foetidus, though incapable of fermenting sugars, resembles so closely the B.C.C. group in the appearance of its colonies on taurocholate lactose agar, that we are inclined to consider it an attenuated member of that group; more especially as we have isolated organisms which have lost their power of gas production after prolonged cultivation.

It is peculiar that though Nos. 25 to 30 resemble each other in almost every respect, we were unable to obtain any growth upon taurocholate lactose agar plates from Nos. 28, 29, and 30.

The colonies of No. 31, B. rhinoscleroma, and No. 48, B. Zopfi, upon taurocolate lactose agar, are indistinguishable from each other.

The remaining organisms call for no further remark.

Now it is obvious, from a consideration of the tables given above, that of all the organisms with which we have worked there are only seventeen which give the complete reaction in taurocholate broth; and of these thirteen are distinctly intestinal.

With regard to the remaining four, we consider:—

1. That as milk is so exposed to contamination, and the B. acidi lactici (HUEPPE) bears such a close relationship to B. coli, it should be placed in the same group.

2. That the B. pneumoniae (FRIEDLÄNDER) will not invalidate the test, because it is a noxious organism, and in the present state of our knowledge can not be said to be non-intestinal.

3. That the B. cloacae (JORDAN), having apparently been isolated only from waters certainly contaminated, may be looked upon as evidence of pollution.

4. That the M. candicans (FLÜGGE) may be neglected, on account of the rarity of its appearance in our work.

It is possible that future research may reveal sources of error or confusion, but up to the present this test has in our hands proved itself simple and reliable.

5

MILK AS A VEHICLE OF TUBERCLE

MILK AS A VEHICLE OF TUBERCLE AND PRESENT LOCAL LEGISLATION IN REGARD TO IT

By E. W. HOPE, M.D., Medical Officer for the City of Liverpool

It is obvious that numerous points are involved in the subject, some of which are difficult to dissociate from questions other than those which concern tuberculosis only. For example, measures taken with the sole object of checking an even more destructive form of disease, viz., diarrhoea, have proved incidentally a safeguard against tuberculosis, whilst, on the other hand, measures directed against tuberculosis have afforded a valuable protection from other forms of disease.

Sterilization of milk possesses one conspicuous advantage, viz., that the application of the safeguard is within the reach of every reasonably prudent and careful household, consequently for ease of application it is beyond any comparison with other preventive measures. The objections to it do not appear to be important, but there are the facts to be reckoned with, that in the lower quarters of every great town there are thousands of families neither prudent nor careful, and also that the population of this country as a rule prefer to take their milk raw. This preference results no doubt partly from thoughtlessness and partly from habit. Young children are trained to take it raw,and the belief is widespread, that if the milk is raised in temperature to say 200° F., or even still nearer the boiling point, it is altered in flavour and constitution, and is of less nutritive and digestive value than when it is given raw; the raw milk in fact is regarded as more nearly approaching the natural milk of the mother.

There is no clinical evidence whatever to show that sterilized or even boiled milk is less nutritious and valuable than raw milk. On the other hand, raw cows' milk, in addition to the risk of tuberculosis, brings many others. The process of milking may involve dirt from a dirty milker, from dirty udders into a dirty milk pail. From this it may be passed through a dirty strainer into a dirty railway can. It is discharged from the railway can into smaller vessels in which it is hawked about the dusty streets, passing through some half-dozen other pots and pans before it reaches the nursery or the table of the consumer, involving a host of possible sources of contamination, not excepting the contamination of Tubercle Bacillus, in fact, it may be safely said, there is no article of food in common use so constantly exposed to contamination, or so susceptible of contamination, as raw milk. The milk, on the

other hand, as Nature intended it to be given, is never once exposed to air, passing directly and at the time of its manufacture in the gland, to the stomach of the young animal, and, apart from the possibility of disease in the gland, is bacteriologically clean and pure.

Sterilization, valuable as it is as a final safeguard against tuberculosis, is after all only an expedient, and must not be put into so much prominence that the importance of the safeguard afforded by keeping the cows healthy is lost sight of, although we cannot take it for granted, in considering the merits of different methods, that essential accessories common to them all will be observed. The one merit of sterilization is that it is an expedient easy of application and presenting few administrative difficulties. Beyond any question the ultimate advantage lies in obtaining the milk from herds free from tuberculosis. It is, in fact, comparable with the advantage of obtaining drinking water from a pure source, instead of taking it from a contaminated one and relying upon purification afterwards. The first aim must be to ensure that the source of the milk is pure ; in other words, that the cows are free from tuberculosis, or if this, under existing conditions of the law and public opinion, is unattainable, that they shall at least be free from any tuberculous disease of the udder, or any tumour or condition of the udder simulating tuberculous disease, or, having regard to difficulties in diagnosis, we may with advantage go even a step further, and demand that the udder in all cows from which milk is taken for human food shall be in a perfectly normal condition.

The main causes of tuberculosis in cows are notorious : close confinement in ill-ventilated, badly-lighted, ill-constructed and dirty cowsheds—defects all as easy to remedy as is removal from the cowshed of the obviously tuberculous animal before it can cause infection of the rest.

In the city of Liverpool about 26,000 gallons of milk are consumed every day ; one-half of it comes from cows, about 6,000 in number, kept within the city, the other half comes from cows kept in the country, and is sent in by rail. Within recent years that part of the milk supply which comes from cows kept within the city has been practically free from tuberculosis. This has been brought about by the sanitation of the cowsheds, adequacy of air, light, and cleanliness, by systematic and frequent inspection of the cows by qualified inspectors with veterinary help, by frequent bacteriological analysis of the samples of milk : these are the measures which have effected this end. I do not say that out of the 6,000 cows in the city there is not a single one affected with tubercle, but merely that there are few with such forms of tuberculous disease as would be likely to contaminate the milk supply.

These methods and this system of inspection were not initiated without difficulty and opposition. There is no opposition now ; every person acquiesces in advantages which have been gained. But there is another aspect to the question. Only one half of the quantity of milk consumed in Liverpool is supplied from the

city, the remaining half comes from the country districts, but, it may be said, if the cows kept in the cowsheds within a great and populous city are healthy, those coming from the sunny meadows of the country, with their fertile pastures and ample land, are free also. Unfortunately, experience does not bear this out, the milk sent in from the country is more frequently tuberculous; thus out of 422 town samples examined during 1899 and 1900, five were tubercular, being a little more than one per cent., but out of 490 country samples taken during the same period, twenty were tubercular, being a trifle over four per cent. How can we protect ourselves against this? A special Act of Parliament applying to a few great towns, including Liverpool, gives special powers to exclude from the city, under a penalty, the milk coming from the country cowsheds in which tuberculous cows are kept under dirty and insanitary conditions. But if it is difficult to deal with and supervise the supply within our own city, it is evidently both costly and difficult to maintain a staff to send, under the special Act of Parliament, to the insanitary and tubercle-ridden cowsheds of the country cowman; but having overcome these difficulties, the broad national question comes in, for, although we succeed in protecting ourselves, what happens with regard to the diseased cows and the diseased milk? The dealer refrains from sending diseased milk to the protected city, but what is there to prevent him from sending his milk for sale and consumption to a district where no special Act of Parliament exists to enable the community to protect itself, or from selling his diseased cows to a dairyman in another locality. This is not the way to secure a supply of milk from herds free from tuberculosis, but there can be little doubt that the action of the great cities will not only protect themselves, but will, to a certain extent, protect the country districts also, and will strengthen the hands of rural sanitary authorities. No doubt the great cities are financially better able to protect themselves; they have their larger and more costly staff, they have their bacteriological laboratories, their veterinary and medical officers, but at best they are but valuable allies to the rural sanitary authorities, and these, after all, must take their own action, since the protection the cities afford them is an indirect vicarious one, and as in cases already alluded to, there is nothing to prevent the cow-keeper from sending his diseased produce to rural districts, after he has been prohibited from sending it to the great cities. Furthermore, the undoubted decline in the proportion of tuberculous milk sent in from the country may really mean that a larger proportion is consumed elsewhere. The subject is quite important enough for a Government Department, *e.g.*, the Local Government Board to take in hand and appoint a special staff to supervise the milk supply and all appertaining to it throughout the country.

It is quite possible to ensure that the milk supply shall come from cows free from tuberculosis. Difficulties, from ignorance, obstruction, and active opposition may be taken for granted, but these must be overcome, and the cow-keeper will

learn in time that his own interests are identical with those of his customers, and by keeping healthy cows in a sanitary condition he will be a gainer in every way.

It is only right to emphasize the fact that during the last year the samples of milk taken at the railway stations on arrival from the country did not appear to be more frequently tubercular than the samples taken from the town. This may indicate one of two things; either a general improvement in the country cowsheds under the stimulus of city action, or as in more cases than one which have come under notice, dairymen, who have been detected in sending in tuberculous milk, have refrained altogether from sending milk to Liverpool, and now send their milk elsewhere. These are points not to be lost sight of.

The Liverpool Corporation Act, 1900, contained, amongst others, the following important clauses, designed to protect consumers of milk from the dangers of tuberculosis:—

17. In this Part of this Act—

'Dairy' shall include any farm, farmhouse, cowshed, milk store, milk shop, or other place from which milk is supplied, or in which milk is kept for purposes of sale:

'Dairyman' shall include any cowkeeper, purveyor of milk, or occupier of a dairy:

'Medical Officer' means the medical officer of health for the city, and includes any person duly authorized to act temporarily as medical officer of health.

18. Every person who knowingly sells or suffers to be sold or used for human consumption within the city the milk of any cow which is suffering from tuberculosis of the udder, shall be liable to a penalty not exceeding ten pounds.

19. Any person the milk of the cows in whose dairy is sold or suffered to be sold or used for human consumption within the city, who after becoming aware that any cow in his dairy is suffering from tuberculosis of the udder, keeps or permits to be kept such cow in any field, shed, or other premises along with other cows in milk, shall be liable to a penalty not exceeding five pounds.

20. Every dairyman who supplies milk within the city, and has in his dairy any cow affected with, or suspected of, or exhibiting signs of tuberculosis of the udder, shall forthwith give written notice of the fact to the medical officer, stating his name and address, and the situation of the dairy or premises where the cow is.

Any dairyman failing to give such notice shall be liable to a penalty not exceeding forty shillings.

21. (1) It shall be lawful for the medical officer or any person provided with and, if required, exhibiting the authority in writing of such medical officer, to take within the city for examination samples of milk produced, or sold, or intended for sale within the city.

(2) The like powers in all respects may be exercised outside the city by the medical officer or such authorized person if he shall first have obtained from a justice, having jurisdiction in the place where the sample is to be taken, an order authorizing the taking of samples of the milk, which order any such justice is hereby empowered to make.

22. (1) If milk from a dairy situate within the city is being sold or suffered to be sold or used within the city, the medical officer or any person provided with and, if required, exhibiting the authority in writing of the medical officer, may, if accompanied by a properly qualified veterinary surgeon, at all reasonable hours, enter the dairy and inspect the cows kept therein; and if the medical officer or such person has reason to suspect that any cow in the dairy is suffering from tuberculosis of the udder he may require the cow to be milked in his presence and may take samples of the milk, and the milk from any particular teat shall, if he so requires, be kept separate and separate samples thereof be furnished.

(2) If the medical officer is of opinion that tuberculosis is caused or is likely to be caused to persons residing in the city from consumption of the milk supplied from a dairy situate within the city or from any cow kept therein he shall report thereon to the Corporation, and his report shall be accompanied by any report furnished to him by the veterinary surgeon, and the Corporation may thereupon serve upon the dairyman notice to appear before them within such time, not less than twenty-four hours, as may be specified in the notice to show cause why an order should not be made requiring him not to supply any milk from such dairy within the city until the order has been withdrawn by the Corporation.

(3) If the medical officer has reason to believe that milk from any dairy situate outside the city, from which milk is being sold or suffered to be sold or used within the city, is likely to cause tuberculosis in persons residing within the city, the powers conferred by this section may in all respects be exercised in the case of such dairy, provided that the medical officer or other authorized person shall first have obtained from a justice having jurisdiction in the place where the dairy is situate an order authorizing such entry and inspection, which order any such justice is hereby empowered to make.

(4) Every dairyman and the persons in his employment shall render such reasonable assistance to the medical officer or such authorized person or veterinary surgeon, as aforesaid, as may be required by such medical officer, person, or veterinary surgeon for all or any of the purposes of this section, and any person refusing such assistance or obstructing such medical officer, person, or veterinary surgeon in carrying out the purposes of this section shall be liable to a penalty not exceeding five pounds.

(5) If in their opinion the dairyman fails to show cause why such an order may not be made as aforesaid the Corporation may make the said order and shall forthwith serve notice of the facts on the county council of any administrative

county in which the dairy is situate and on the Local Government Board, and, if the dairy is situate outside the city on the council or borough or county district in which it is situate.

(6) The said order shall be forthwith withdrawn on the Corporation or their medical officer being satisfied that the milk supply has been changed or that it is not likely to cause tuberculosis to persons residing in the city.

(7) If any person after any such order has been made supplies any milk within the city in contravention of the order or sells it for consumption therein he shall be liable to a penalty not exceeding five pounds, and if the offence continues, to a further penalty not exceeding forty shillings for every day during which the offence continues.

(8) A dairyman shall not be liable to an action for breach of contract if the breach be due to an order under this section.

(9) The dairyman may appeal against an order of the Corporation under this section or the refusal of the Corporation to withdraw any such order either to a petty sessional court having jurisdiction within the city or at his option, if the dairy is situate outside the city, to the Board of Agriculture who shall appoint an officer to hear such appeal. Such officer shall fix a time and place of hearing within the city and give notice thereof to the dairyman and the town clerk not less than forty-eight hours before the hearing. Such officer shall for the purposes of the appeal have all the powers of a petty sessional court.

(10) The Board of Agriculture may at any stage require payment to them by the dairyman of such sum as they deem right to secure the payment of any costs incurred by the Board of Agriculture in the matter of the appeal.

24. Offences under this Part of this Act may be prosecuted and penalties may be recovered by the Corporation before a petty sessional court having jurisdiction in the place where the dairy is situate or the offence is committed and not otherwise.

THE EXCRETORY AND TUBERCULAR CONTAMINATION OF MILK

THE EXCRETORY AND TUBERCULAR CONTAMINATION OF MILK

By RUBERT BOYCE

As the result of analysis which have been made in the Laboratory during the last eighteen months, namely of 1,020 samples of milk, and 733 samples of water, it will be seen at a glance, that, as compared with water, milk is a highly contaminated substance.

The average number of bacteria in the water in Liverpool is 28 in 733 samples. The B. coli was found only seven times, and a gas forming anaerobe* in none of the samples. This water from its source to its supply to the consumer is under the strictest possible control. Inspectors are on the watershed, and are stationed along the pipe line; the filtration beds and reservoirs are under strict supervision and bacteriological investigations are made daily. The result is a pure supply, and no fear of infection by means of it.

The contrast between milk and water is very remarkable, and unmistakably shows that the milk supply of the country constitutes a very strong element of danger. For example, leaving out the total number of bacteria per c.c. which is so great that it is not to be compared with water, it will be seen in the Tables appended that B. coli is present 205 times in 1,026 samples or 19·09 per cent., and that a gas forming anaerobe is present 113 times. This means that owing to the crude methods of collecting and dealing with milk that excretory contamination is liable to occur to the extent of 19·09 per cent. If water contained such an average percentage its use would be immediately forbidden. How this extraordinary degree of contamination can take place is only too well known to those who have entered into the question of milk production. The significance of the contamination is obvious. If B. coli is present, so may pathogenic organisms, such as the B. typhosus. The B. coli not only indicates dirt and intestinal contamination, but it also may become harmful in itself and give rise to intestinal inflammations. It shows that if such neglect of the rules of cleanliness can take place in the shippon, in all probability the health of the animal giving the milk is equally neglected. And what do we find? Of the 1,026 samples 27 are tubercular, giving a percentage of 2·6 per cent.

In the majority of these cases the udder was obviously diseased, and yet the milk of the animal was consumed. If neglect such as this can occur in the case of tuberculosis of the udder, it is quite certain that there are animals suffering from

* Obtained by heating the milk at 80 C for fifteen minutes, and incubating anaerobically. As in all cases an inoculation into the guinea pig has not been made we cannot say that the organism is the B. enteritidis sporogenes.

generalized tuberculosis and other diseases, the milk of which is sold to the public although it is dangerous to the health of man. This disregard of care would not be tolerated in any other article of consumption, as far as I am aware.

If intelligent supervision is rendering the water supply of large towns above suspicion, surely we have a right to demand, and to insist upon, similar care in the case of the milk supply. As it is well known, milk dealers have recognized the danger of raw milk, and have long since taken to condensing and sterilizing and adding preservatives. In certain cases this departure has been a great boon, but it has been abused; the condensed milk is not sterile, we do not know by its constituents whether bacterial products are there or not. Sterilized milk, unless the source is known, is no guarantee that the milk was pure to commence with. The addition of preservatives is in the vast majority of cases a cloak of the worst description, and ought to be as strenuously forbidden as it is in Germany. None of these methods strike at the root of the evil. Intelligent supervision at the place of production and during distribution is necessary. If the milk producers themselves do not brace themselves up and see that it is to their advantage to improve their supply and its keeping properties, municipal authorities must do so. In one or two large cities the municipality has undertaken the task in earnest and with most striking results. I quote from Liverpool: of 372 samples of milk taken from the Liverpool shippons, 7 were tubercular, 41 contained B. coli, and 27 a gas-forming anaerobe; of 414 railway-borne samples and therefore coming to Liverpool from a distance, 11 were tubercular, 105 contained B. coli, and 54 the gas-forming anaerobe. This shows that supervision in the case of the town shippons is beginning to have its effect, and that the milk has become purer than that from the country. The country-produced milk, with all the advantages which the country offers, is bad, for there is an almost total lack of organized supervision, and gallons of contaminated milk are allowed to find their way into vast populations to swell the chronic disease rate, the infant mortality, and the expenditure.

But why, finally, should this work altogether be left to Corporations. Cannot the milk producers in a county, for instance like Cheshire, establish a laboratory where everything relating to milk may be studied; where the best means of diagnosing tubercle may be investigated, where the purity of the milk may be checked, and where the best means of cleansing and sending out milk may be worked out. In Germany, the co-operation between the butchers and the authorities is very striking, and the butcher gains, for very little is wasted.

The following case which has recently come under my notice shows the intolerable ignorance which still exists as regards the treatment of milk. Samples of milk were taken separately from the cows in a shippon, but instead of the milker washing his hands between each milking, he spits on them. Two of the samples of milk are returned as tubercular, by the guinea pig inoculation test, but subsequently

only one cow is proved to be suffering from tubercle. But it is obvious that the milker with his hands contaminated with the bacilli from the diseased cow may have infected the outside of the teats of any of the other cows, and so have inoculated the second sample. It is probable that this may be one of the ways in which primary tuberculosis of the udder is produced. Again, the milker might have been tuberculer himself, and might have infected the cow by this most objectionable habit, which, I trust, is exceptionable, This points to one other conclusion, and that is, if a tubercular cow is found in a shippon, everything, udders, pails, walls, floors, should be thoroughly disinfected as in the case of anthrax. We run more danger from tubercle than anthrax and proper precautions should be taken. One tubercular cow may infect a whole shippon, and therefore the milk supply from it.

Tables shewing the comparative frequency of contamination in Milk, Water, and Shell Fish

MILKS

Source	Number of samples examined	Bacillus coli communis	Gas forming anaerobe	Number of tubercular milks
Town	372	41	27	7
Railway	414	105	54	11
'B'	105	32	3	5
'S'	4	2	...	...
'W'	125	22	28	4
'W'	6	3	1	...
Totals ...	1,026	205	113	27

WATER

Number of samples examined	Number of bacteria per cc.	Bacillus coli communis	Gas forming anaerobe
733	28	7	0

SHELL FISH

Number of samples examined	Bacillus coli communis	Gas forming anaerobe
217	42	50

REPORT TO THE MEDICAL OFFICER OF THE BACTERIOLOGICAL EXAMINATIONS MADE FOR THE CITY OF LIVERPOOL DURING THE YEAR 1900

REPORT TO THE MEDICAL OFFICER OF THE BACTERIOLOGICAL EXAMINATIONS MADE FOR THE CITY OF LIVERPOOL DURING THE YEAR 1900

By RUBERT BOYCE

BACTERIOLOGICAL EXAMINATIONS AND ANALYSES

The work has comprised :—

(*a*) Examination of food stuffs of various kinds.

(*b*) Regular examination of water supplied to the City.

(*c*) Examinations into suspected cases of rabies, anthrax, glanders, etc.

(*d*) Examination for diagnostic purposes in suspected cases of diphtheria, typhoid fever, tubercular sputum, etc.

(*e*) Special investigations.

Every food-stuff and every sample of water is analysed for the presence of (1) Bacillus coli ; (2) Bacillus enteritidis sporogenes.

Every sample of milk, cream, butter, margarine, and cheese, is, in addition, examined for the presence of the Bacillus tuberculosis by inoculation.

In every sample of water the number of bacteria present in the cubic centimetre is also noted.

To facilitate these operations special apparatus has been constructed in the laboratory, and many of the operations have been simplified by their use.

With regard to (*a*) the total number of samples of food-stuffs taken for bacteriological examinations during the year 1900 were as follows :—

1,067 Foods
101 Samples of Water
39 Miscellaneous examinations

In addition a very large number of bacteriological examinations were made of suspected Tubercular, Typhoid, and Diphtheria cases for the medical practitioners of the district.

Five hundred and fifty-six Typhoid and Diphtheria examinations.

The following is a list of food-stuffs examined:—

Sample	Number	Sample	Number
Bloaters (Tinned)	1	Oleo	1
Bloater Paste	9	Orange Butter	2
Bovril	1	Oysters (Tinned)	2
Brawn	3	Periwinkles	22
Bottled Plums	2	Polony	3
Boiled Rabbit (Tinned)	1	Preserved Tomatoes	11
Bottled Gooseberries	1	„ Pineapple	6
Black Treacle	1	„ Peaches	1
Butter	21	„ Apricots	2
Cockles	35	„ Peas	5
Cream	8	Picalilli	1
Condensed Milk	25	Pineapple Butter	1
Cheese	13	Pudding	1
Crab Paste	1	Potted Shrimps	3
Chutney	4	„ Beef	2
Chicken, Ham, and Tongue ...	2	„ Lobster	2
Chicken and Ham	4	Pork, Boiled and Smoked ...	1
Cream Cheese	1	Pork Pie	3
Dripping	4	Potted Tongue (Tinned) ...	2
Extract of Coffee	4	„ Beef (Tinned)	7
Flour	6	„ Ham	6
Fresh Herrings	1	„ Mixed Game	1
Fluid Beef	3	Sauces	21
Fruit Cream	1	Sausages	16
Fruit Syrups	2	Sago	1
Food Jelly	1	Sardines (Tinned)	20
Golden Syrup	1	Sterilized Milks	9
Honey	4	Salmon (Tinned)	13
Herrings and Tomato Sauce ...	1	Sausages (Tinned)	1
Infants' Food	1	Sweetmeats	2
Jams	18	Sugar	1
Jellies	14	Suet	1
Lobster (Tinned)	4	Tapioca	1
Lard	6	Turkey and Tongue	3
Lemon Curd	7	Veal and Ham	2
Lemon Cheese	1		
Margarine	14		1,067
Mussels	32	Water	101
Margarine (Tinned)	1	Typhoid and Diphtheria ...	556
Mince Meat	2	Miscellaneous examinations ...	39
Milk	560		
Oysters	65	Total ...	1,763
Oatmeal	5		

MILK ANALYSES FOR THE YEAR

The total number of milks examined was five hundred and sixty. These were examined for the presence of:—

1. The Bacillus tuberculosis
2. The Bacillus coli
3. The Bacillus enteritidis sporogenes
4. Other bacteria

The Bacillus tuberculosis indicates that the animal from which the milk was taken was tubercular, or that the pails into which the milk was received, or the hands of the milker, were infected from previous contact with a diseased cow.

The Bacillus coli indicates contamination with dirt, of an intestinal origin, or possibly that the cow was suffering from inflammation of the udder.

The Bacillus enteritidis sporogenes indicates dust or intestinal contamination.

Presence of the Tubercle Bacillus

Of the five hundred and sixty samples examined for tubercle, one hundred and five guinea pigs died before the tubercular test was completed, leaving four hundred and fifty-five samples for the completion of the investigation. *Of this number nine proved tubercular*, five were found in *railway* borne milks, and four in *town milks.*

The greater frequency of tubercle in railway-borne milks was also noted last year. It is a very serious matter that tubercle is still so wide-spread in milk. When it is remembered that one tubercular cow may be the means of infecting the milking utensils, the hands of the milker, and even the teats of the other healthy animals, regulations to deal with infected animals cannot be too stringent.

Presence of the Bacillus enteritidis sporogenes and the Bacillus coli

The Bacillus enteritidis sporogenes was found twenty-six times in two hundred and fifty-five town samples of milk, and forty-two times in three hundred and five railway-borne samples.

The Bacillus coli was present fifteen times in the town milks, and forty times in the railway milks.

This is an exceedingly interesting and important result, for it shows that less care is taken in handling the country milk, and, therefore, that contamination much more frequently occurs. Bacillus enteritidis sporogenes appears most common in March and April; Bacillus coli in November and December.

In the case of the railway-borne milk, the Bacillus coli was most abundant in December, and this may indicate that, in addition to dirt contamination, a possible other source of the coli was inflammation of the udder.

With regard to the relationship of the Bacillus coli to the Bacillus enteritidis sporogenes, it has been found that very frequently the two organisms do not occur together. The significance of this is important as throwing light upon the significance of the Bacillus enteritidis sporogenes as an index of pollution. Where the Bacillus coli and Bacillus enteritidis sporogenes occur together this would be strong evidence that the Bacillus enteritidis sporogenes was of recent intestinal origin. But in a very large number of cases the Bacillus enteritidis sporogenes occurs alone. In these cases it is very hard to say what importance is to be attached to its presence, and unless an inoculation test of the virulence of the Bacillus is made, it would be impossible to say whether the Bacillus is enteritidis sporogenes or butyricus.

When dealing with a very large number of food-stuffs, it very greatly increases the work if the pathogenicity of the Bacillus which is isolated has to be tested each time.

Table showing the frequency with which the Bacillus coli and Bacillus enteritidis sporogenes occur alone and together in five hundred and sixty samples of milk analyzed.

Date	Number of Samples	Bacillus coli alone	Bacillus enteritidis alone	Together
January	45	5	1	...
February	45	1	3	1
March	55	2	11	3
April	41	3	13	3
May	50	4	9	3
June	48	...	3	2
July	44	1	6	...
August	45	1	5	...
September	44	...	4	...
October	57	4	2	2
November	40	11	2	...
December	46	15	...	...

TABLE SHOWING THE TOTAL NUMBER OF MILKS WHICH WERE EXAMINED DURING 1900 FOR TUBERCLE, BACILLUS COLI COMMUNIS, AND BACILLUS ENTERITIDIS SPOROGENES

Month	Town	Bac. Coli Com.	Bac. Ent. Spor.	Rail.	Bac. Coli Com.	Bac. Ent. Spor.	Hospital	Bac. Coli. Com.	Bac. Ent. Spor.	Total Number	Number of Tubercular Milks
January	21	3	...	20	2	...	4	...	...	45	1 Town
February	21	...	1	20	2	3	4	...	...	45	...
March	28	3	7	20	2	6	7	...	1	55	1 Railway
April	17	1	8	19	3	8	5	2	...	41	1 Town
May	20	5	4	26	2	8	4	...	...	50	1 Town
June	24	...	...	18	2	5	6	...	...	48	1 Town
July	20	1	1	20	...	4	4	...	1	44	...
August	20	...	2	20	1	1	5	...	2	45	2 Railway
September	22	...	2	16	...	2	6	...	...	44	...
October	31	...	...	21	6	4	5	1	...	57	2 Railway
November	10	2	1	25	7	1	5	2	...	40	...
December	21	...	...	20	13	...	5	2	...	46	...

TABLE SHOWING THE TOTAL NUMBER OF MILKS EXAMINED BACTERIOLOGICALLY FOR TUBERCLE BACILLI FROM AUGUST, 1896, TO 31ST DECEMBER, 1900

Year	Total Number of Samples taken	Town Samples			Country Samples		
		Number taken	Tubercular	Percentage Tubercular	Number taken	Tubercular	Percentage Tubercular
1896	119	83	4	4·8 per cent.	36	5	14·0 per cent.
1897	150	63	4	6·3 „	87	5	5·0 „
1898	112	84	7	8·3 „	28	5	17·9 „
	381	230	15	6·5 per cent.	151	15	10·0 per cent.
1899	352	167	1	0·6 per cent.	185	15	8·1 per cent.
1900	560	255	4	1·5 „	305	5	1·6 „
	912	422	5		490	20	
Totals ...	1,293	652	20	3·0 per cent.	641	35	5·3 per cent.

RESULTS OF ANALYSES OF BUTTER, CREAM, STERILIZED MILK, CONDENSED MILKS, CHEESE, LARD, AND MARGARINE

Butter. Twenty-one samples were analysed and the tubercle bacillus found in one case. If tubercle is present in milk, it can also be present in butter, cream, and margarine, and, therefore, the finding it in these food-stuffs is a further reason for increasing the vigilance of dairy supervision.

Creams. Eight samples of cream were examined and the Bacillus coli found twice and the Bacillus enteritidis sporogenes once.

Sterilized Milks. Of the nine samples examined one was found not to be sterile. The sterilization of milk is difficult on account of the presence of spore-bearing bacilli, the resistance of which to heat is very considerable.

Condensed Milks. Twenty-five samples were examined and the great majority were not sterile. There is no doubt that condensed milk is a most unsatisfactory product. Bacteria are usually present, and the milk, which was originally condensed, might have contained various products of the decomposition of bacteria. These products are masked subsequently by the large quantity of sugar present, but their irritant properties are not destroyed.

Cheese. Thirteen samples were examined. In one case the Bacillus coli was present, and in another sample the Bacillus enteritidis sporogenes. The probability is that in cheese, organisms like the Bacillus coli and Bacillus tuberculosis, which might have been originally present in the milk from which the cheese was made, tend to die out in the process of fermentation.

Lard and Margarine. Twenty-one samples were examined. No tubercle was found, and the Bacillus enteritidis in only one sample of margarine.

Bacteria present in Shell Fish. Some kinds of shell fish, like milk and milk products, are for the most part eaten uncooked ; they are in consequence liable to convey infection if they become contaminated with pathogenic bacteria. Contamination may occur in the transit and storing of the shell fish, but more especially in the collecting grounds. It is not uncommon to find that sewage has access to oyster, mussel, and cockle beds. One hundred and fifty-four samples were examined for evidence of the Bacillus coli and Bacillus enteritidis sporogenes. The Bacillus coli was present seventeen times, the Bacillus enteritidis thirty-seven times. The Bacillus coli was more frequently present in oysters and mussels, the Bacillus enteritidis in periwinkles and cockles. Thus again, as in the case of the milks, there is little uniformity between the occurrence of these two bacilli. It is fortunate that Bacillus coli is not more abundant in shell fish in Liverpool, but no efforts must be spared to make the collecting grounds above suspicion of sewage contamination. In the case of cockles and mussels, this is difficult, as they are often taken from the mouths of estuaries where pollution unfortunately occurs to a great extent owing to the discharge of crude sewage.

Sausages. As in the case of sterilized milk, condensed milk, and raw foods generally, so in the case of sausages it is all important that the ingredients should be pure, otherwise the spice simply masks the bacterial changes, and does not destroy the ptomaines or indeed injurious bacteria. Seventeen samples were examined, and the Bacillus coli obtained in six samples and the Bacillus enteritidis in fourteen samples.

Tinned Meats, Fruits, and Vegetables. Ninety-four sampies were examined, and in no case was either the Bacillus coli or Bacillus enteritidis sporogenes found. A few samples were not sterile.

Pastes and Potted Meats. In only one case out of eleven samples was the Bacillus enteritidis sporogenes found. Nine out of eleven were not sterile.

Cereals. Considerable interest attaches to the bacterial examination of these articles, because they are very liable to dust contamination. Thirteen samples were examined, of which four showed evidence of the Bacillus enteritidis sporogenes. No coli was found.

Jams. Jams have shown a freedom from dangerous or danger indicating bacteria. Many are sterile. Those which are not sterile only contain a few bacteria. There is no doubt that the greatest care must be used in the boiling and subsequent distribution of the jam into pots to ensure sterility and keeping properties.

The following is a summary of the chief investigations and analyses, together with references to the methods employed:—

1. The Injurious Effects of Foods and Beverages preserved with Boracic and Salicylic Acids

To test the injurious action of these preservatives, kittens, three weeks old, were fed with milk containing these preservatives in the proportion in which they were found in articles of diet. It will be seen from the table that the kittens fed on boracized milk from May 25 to June 2 failed not only to gain weight, but actually lost considerably in many cases. The control kittens, on the other hand, fattened in the usual manner. Further, the boracized kittens suffered in health, and were subject to diarrhoea. On June 8, a pure milk diet was substituted for the boracized milk, and the kittens rapidly gained in weight. These experiments confirm those which had been made in the previous year.

Further experiments made by Dr. Grünbaum in this laboratory have shown that the addition of borax to milk to the extent of 0·4 per cent. by precipitating the calcium is sufficient to inhibit the action of the rennet ferment, whilst at the same time the inhibiting effect on the growth of pathogenic micro-organisms is practically *nil.* On the other hand, keeping milk cooled to 40 deg. F. almost entirely stops the growth of the bacteria.

Both the feeding and digesting experiments show that boracic acid in milk is injurious, and ought not to be added.

BORACIC ACID EXPERIMENTS

Kittens fed with Boracized Milk containing about 82 grains to the pint			Control Kittens fed with Pure Milk		
Date 1900	Kitten	Weighed	Date 1900	Kitten	Weighed
May 26	No. 1	822 grms.	May 26	No. 1	550 grms.
„	„ 2	602 „	„	„ 2	595 „
„	„ 3	715 „	„	„ 3	710 „
„	„ 4	765 „	„	„ 4	530 „
„	„ 5	620 „	„	„ 5	570 „
May 30	No. 1	715 grms.	May 30	No. 1	624 grms.
„	„ 2	602 „	„	„ 2	616 „
„	„ 3	702 „	„	„ 3	818 „
„	„ 4	751 „	„	„ 4	624 „
„	„ 5	540 „	„	„ 5	632 „
June 2	No. 1	777 grms.	June 2	No. 1	670 grms.
„	„ 2	580 „	„	„ 2	648 „
„	„ 3	717 „	„	„ 3	850 „
„	„ 4	755 „	„	„ 4	680 „
„	„ 5	500 „	„	„ 5	643 „

On June 8, the Kittens which had been fed with Boracized Milk were changed to a diet of Pure Milk

Date 1900	Kitten	Weighed	Date 1900	Kitten	Weighed
June 8	No 1.	880 grms.	June 8	No. 1	678 grms.
"	" 2	720 "	"	" 2	655 "
"	" 3	840 "	"	" 3	890 "
"	" 4	832 "	"	" 4	700 "
"	" 5	510 "	"	" 5	590 "
June 15	No 1.	800 grms.	June 15	No. 1	590 grms.
"	" 2	860 "	"	" 2	615 "
"	" 3	895 "	"	" 3	1,000 "
"	" 4	800 "	"	" 4	737 "
"	5 "	505 "	"	" 5	636 "
June 22	No 1.	Missing	June 22	No. 1	682 grms.
"	" 2	1,050 grms.	"	" 2	695 "
"	" 3	1,080 "	"	" 3	1,010 "
"	" 4	790 "	"	" 4	980 "
"	" 5	600 "	"	" 5	690 "

In the case of the salicylic acid experiments, one kitten fed on salicylized milk increased in weight from October 23, when the experiments were commenced, till December 4, when the experiments ended. A second kitten decreased in weight, and died on December 16. The third kitten at first increased in weight, and then began to lose, and died on November 27. Of the control kittens, the first and second increased in weight from the commencement to the end of the experiments; the third died too soon after the commencement of the observations for any deductions to be made. These experiments show that salicylic acid has an injurious effect, though less marked than boracic acid, but further research is necessary.

The injurious effects of formalin were fully dealt with in last year's Annual Report.

2. Experiments and Observations upon the significance of the Bacillus enteritidis sporogenes

Like the Bacillus coli, this organism is systematically looked for in waters and food-stuffs. Dr. Klein has laid considerable stress upon its presence, as he considers that it is capable of causing diarrhoea much in the same way as the Bacillus coli.

During the year much evidence has been accumulated to show its distribution in food-stuffs, and special investigations have been made to determine its significance. Attention was especially drawn to this organism by a case of poisoning which was thought might be due to eating diseased salted fish. Examination of the dried fish showed the presence, amongst other bacteria, of the Bacillus enteritidis sporogenes. Subsequent examinations of numerous examples of dried fish, however, showed that this organism was normally present. A series of analyses of foods liable to dust contamination was then made, viz., wheat, barley, oats, oatmeal, flour, rice, cornflour, clovers, grasses, etc. Sixty samples were examined, and forty-one gave an enteritidis-like growth in milk, and thirty were fatal to guinea pigs when inoculated, and eleven produced an inflammatory reaction.

Further research demonstrated that the bacillus was widespread. The pathogenicity of the bacillus isolated was tested, in order to make certain that the bacillus isolated was that described by Dr. KLEIN.

The observations of the year's analyses show that the organism is abundant in milk and other food-stuffs, and our conclusions are that the Bacillus enteritidis sporogenes is much more widespread than the Bacillus coli, owing no doubt to its power of spore formation, and that, therefore, although originally derived from the intestine, its presence in a food is not of the same significance as that of the Bacillus coli. With regard to its pathogenicity in animals there is no doubt, but in man it is like the Bacillus coli, common to the intestine. It may be that certain forms of diarrhoea are due to an increased virulence of this organism in the intestine as in the case of diarrhoea associated with Bacillus coli, but further evidence of this is wanted.

3. EXPERIMENT TO DEMONSTRATE THE SIGNIFICANCE OF THE BACILLUS COLI

This organism is looked for in all samples of foodstuffs and water where bacteria are known or suspected to be present. The reason for this is that it is considered by many to be evidence of sewage contamination. In all the analyses it is therefore stated whether it is present or absent, and the result is that during the past twelve months a mass of evidence shows that the Bacillus coli indicates recent pollution or contact with inflammatory discharges.

Stream and rivulets, not obviously polluted, showed an absence of this organism in the quantities analysed. Sewage and sewage effluents, on the other hand, or streams near human habitations, showed the presence of the Bacillus coli. It has a very low degree of resistance, and soon perishes outside the alimentary tract. This was strikingly demonstrated in the roadways. If the season was dry and the roads dusty, the Bacillus coli was absent or very scanty in the dust. On the other hand, in the gutters along the side of the roads, which are usually moist and often receive garbage, the Bacillus coli were very numerous. Although the Bacillus coli

is normally found in the intestine of man and animals, and, therefore, cannot be said to be under these circumstances harmful, nevertheless cases do occur in which marked diarrhoea is found associated with great development of this organism in the intestine. Such cases of diarrhoea often occur in epidemic form, and the evidence is that under certain circumstances the Bacillus coli may become pathogenic, and produce inflammation in the alimentary tract.

Distribution of Tuberculosis. Dr. Elliott conducted an interesting enquiry into the distribution of tuberculosis in Liverpool and the infectivity of houses in which patients have recently died. He examined the dust in four out of ten houses in which deaths had occurred seven to fourteen days previously, and found, by inoculation in the guinea pig, that the tubercle bacillus was present in one of them. In this infected house there had been carelessness in the disposal of the sputa, and cleanliness had not been observed during and after the patient's death. This is a very important observation, for it shows the danger of the consumptive's room not only during his illness but for some considerable time afterwards, and it also shows the value of the disinfection and cleansing carried out by the disinfecting staff when cases of phthisis are notified.

Plague investigations. During the year numerous rats and several suspected cases of Plague were examined by Dr. Balfour Stewart for the presence of the Plague bacillus, but none was found. To be ready in case of any emergency a stock of vaccine was prepared and kept in the laboratory, and, although no occasion arose in Liverpool for its use, it was supplied to other towns in England where cases had occurred. A large demand also arose for it owing to the outbreak in South Africa, and the total quantity supplied to municipalities, private individuals, the Colonial and War Offices, amounts to seventy thousand doses. At the present time a very large quantity is available for immediate use in case of any emergency. *Nature of the vaccine:* The vaccine is prepared after Haffkine's method, and consists of a sterilized broth cultivation of the virulent plague bacillus. It is put up in sterilized bottles containing a definite number of doses, and is most carefully sealed.

Investigation of 'Pink Eye' in Horses. A severe epidemic of this disease broke out during the year amongst the horses of the Corporation and in private stables.

Having failed to obtain evidence of an organism in the horses, numerous examinations were made of the discharges from the eyes and nose, and of all the organs of horses which were slaughtered whilst suffering from the disease. The organs were examined immediately after death, and included the nasal cavity, trachea conjunctiva, liver, spleen, kidneys, subcutaneous tissues, and heart. From the mucous membranes a characteristic bipolar Bacillus was isolated in large numbers in every case. The Bacillus was pathogenic to guinea pigs, producing fatal results or extensive oedema. This Bacillus was common to all the cases of Pink Eye ; it was

abundant not only in the discharges but far back in the nasal cavity when the head of the horse was opened immediately after death, and it was pathogenic. From its cultural properties it appears to be a member of the Bacillus coli group. Without further observations it would be impossible to state whether this virulent coli form was the cause of the disease. The inquiry will be continued if another outbreak arises.

RABIES

Twenty dogs were examined for rabies, but fortunately in no case was rabies shown to be present.

Date	Result of Inoculation.
January 27	Not Rabies
February 28	do.
March 8	do.
March 9	do.
March 10	do.
April 7	do.
April 9	do.
April 20	do.
June 5	do.
June 11	do.
June 21	do.
July 5	do.
July 5	do.
July 15	do.
July 24	do.
July 31	do.
September 13	Rabbit died Sept. 21
September 21	Not Rabies
October 2	do.
November 6	Inoculation unsuccessful

BACTERIOLOGICAL ANALYSES OF CASES OF TYPHOID AND DIPHTHERIA IN THE CITY FEVER HOSPITALS

During the year the fever hospitals have availed themselves of the facilities of the Municipal Bacteriological Department, and five hundred and fifty-one specimens have been examined.

The following is a summary of the results :—Three hundred and thirty-six cases of diphtheria, two hundred and fifteen typhoid, four malaria, and one tuberculosis.

Of three hundred and thirty-six cases of diphtheria—

158 were positive
141 were negative
23 were no growth
14 were suspicious

336 total

Of two hundred and fifteen cases of typhoid—

124 gave a positive reaction
79 gave a negative reaction
7 were suspicious
5 were not examined for various reasons

215 total

No plasmodium malariae in any of the malaria specimens.

No tubercle bacilli were found in the specimen of sputum.

WATER ANALYSES

All the samples of water have been systematically examined for the presence of the Bacillus coli and the Bacillus enteritidis sporogenes, as well as for the total number of bacteria. The quantity of water used for each analysis has been one cubic centimetre.

The following are the sources which have been examined :—

Fortnightly Examinations—

Ashton Hall Tap.

Monthly Examinations—

PRESCOT— { Lake Vyrnwy Water; Rivington Water; The Mixed Water }

WELLS— { Green Lane Well; Windsor Well; Dudlow Lane Well }

The results show that the Bacillus coli has not been found present in any sample to the one cubic centimetre used.

The average number of Bacteria present in :—

1. Ashton Hall Water . . =19·9 per c.c.
2. Vyrnwy Aqueduct . . =19·6
3. Rivington Aqueduct . . =12·16
4. Green Lane Wells . . =81·0
5. Windsor Well . . =52·0
6. Dudlow Lane Well . . =63·9

Ashton Hall—Fortnightly Samples

Source	Date, 1900	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
Ashton Hall	Jan. 10	10-30 a.m.	10-37 a.m.	39	Absent	Absent
„	Jan. 23	2-30 p.m.	3-0 p.m.	16	„	„
„	Feb. 9	2-5 p.m.	4-30 p.m.	19	„	„
„	Feb. 20	2-15 p.m.	21st 10-30 a.m.	25	„	„
„	Mar. 10	9-30 a.m.	11-40 p.m.	6	„	„
„	Maa. 28	3-30 p.m.	5-0 p.m.	large No.	„	„
„	April 17	10-30 a.m.	11-0 p.m.	10	„	„
„	April 25	10-38 a.m.	11-40 a.m.	13	„	„
„	May 11	3-30 p.m.	5-0 p.m.	6	„	„
„	May 25	2-0 p.m.	3-0 p.m.	20	„	„
„	June 8	3-0 p.m.	4-0 p.m.	3	„	„
„	June 21	3-7 p.m.	4-0 p.m.	41	„	„
„	July 5	3-0 p.m.	3-30 p.m.	29	„	„
„	July 20	10-20 a.m.	11-30 p.m.	12	„	„
„	Aug. 4	9-40 a.m.	11-0 a.m.	30	„	„
„	Aug. 11	11-0 a.m.	12-15 p.m.	22	„	„
„	Sept. 12	11-10 a.m	11-30 a.m.	5	„	„
„	Oct. 1	10-30 a.m.	11-0 a.m.	23	„	„
„	Oct. 9	10-30 a.m.	4-30 p.m.	10	„	„
„	Oct. 28	10-30 a.m.	11-0 a.m.	16	„	„
„	Nov. 5	10-30 a.m.	11-30 a.m.	73	„	„
„	Nov. 24	11-0 a.m.	11-40 a.m.	13	„	„
„	Dec. 7	4-10 p.m.	5-0 p.m.	9	„	„
„	Dec. 29	10-40 a.m.	11-30 a.m.	18	„	„

Green Lane Wells—Monthly Samples

G. Holt Well	Jan. 23	1-13 p.m.	3-0 p.m.	50	Absent	Absent
„	Feb. 20	12-55 p.m.	21st 10-30 a.m.	48	„	„
„	Mar. 27	9-15 a.m.	6-30 p.m.	26	„	„
„	April 28	2-25 p.m.	5-5 p.m.	17	„	„
„	May 25	11-25 a.m.	3-0 p.m.	6	„	„
„	June 22	2-15 p.m.	4-0 p.m.	11	„	„
„	July 20	9-5 a.m.	11-30 a.m.	48	„	„
„	Aug. 8	10-15 a.m.	3-50 p.m.	114	„	„
„	Sept. 20	9-8 a.m.	11-0 p.m.	32	„	„
„	Oct. 12	1-30 p.m.	4-30 p.m.	34	„	„
„ „	Nov. Dec.	Samples not taken, engines not working.				
J. Holmes Well	Jan. 23	1-15 p.m.	3-0 p.m. Gelatine plate broken.			
„	Feb. 20	1-0 p.m.	21st 10-30 a.m.	440	Absent	Absent
„	Mar. 27	9-13 a.m.	6-30 p.m.	62	„	„
„	April 28	2.30 p.m.	5-5 p.m.	46	„	„
„	May—Samples not taken, engines not working.					
„	June 22	2-15 p.m.	4-0 p.m.	112	„	„

Source	Date	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
J. Holmes Well	July 20	9-5 a.m.	11-30 a.m.	40	Absent	Absent
„	Aug. 8	10-20 a.m.	3-50 p.m.	104	„	„
„	Sept. 20	9-11 a.m.	11-0 a.m.	240	„	„
„	Oct. 13	9-15 a.m.	11-30 p.m.	5	„	„
„	Nov. } Samples not taken, engines not working.					
„	Dec. }					

Dudlow Lane—Monthly Samples

Dudlow Lane	Jan. 23	1-38 p.m.	3-0 p.m.	22	Absent	Absent
„	Feb. 20	1-20 p.m.	10-30 p.m.	240	„	„
„	Mar. 17	9-38 a.m.	6-30 p.m.	14	„	„
„	April 28	3-15 p.m.	5-5 p.m.	63	„	„
„	May 25	11-55 a.m.	3-0 p.m.	12	„	„
„	June 22	2-40 p.m.	4-0 p.m.	17	„	„
„	July 20	9-35 a.m.	11-30 a.m.	70	„	„
„	Aug. 8	9-15 a.m.	3-50 p.m.	20	„	„
„	Sept. 20	9-40 a.m.	11-0 a.m.	204	„	„
„	Oct. 12	2-15 p.m.	4-30 p.m.	10	„	„
„	Dec. 7	2-45 p.m.	5-0 p.m.	31	„	„

Windsor Well—Monthly Samples

Windsor Well	Jan. 23	2-10 p.m.	3-0 p.m.	16	Absent	Absent
„	Feb. 20	1-50 p.m.	10-30 a.m.	84	„	„
„	Mar. 27	10-0 a.m.	6-30 p.m.	15	„	„
„	April 28	3-55 p.m.	5-5 p.m.	43	„	„
„	May 25	12-15 p.m.	3-30 p.m.	44	„	„
„	June 22	3-0 p.m.	4-0 p.m.	64	„	„
„	July 20	9-55 a.m.	11-30 a.m.	75	„	„
„	Aug. 8	8-30 a.m.	3-50 p.m.	18	„	„
„	Sept. 20	10-15 a.m.	11-0 a.m.	126	„	„
„	Oct. 12	3-0 p.m.	4-30 a.m.	67	„	„
„	Nov. 8	1-40 p.m.	4-0 p.m.	38	„	„
„	Dec. 7	1-30 p.m.	5-0 p.m.	35	„	„

Prescot—Mixing Well—Monthly Samples

Mixing Well	Jan. 23	4-50 p.m.	6-45 p.m.	17	Absent	Absent
„	Feb. 20	5-53 p.m.	10-30 a.m.	—	„	„
„	Mar. 28	2-10 p.m.	5-0 p.m.	40	„	„
„	April 24	2-8 p.m.	25th 10-30 p.m.	12	„	„
„	May 23	2-15 p.m.	4-0 p.m.	13	„	„
„	June 19	1-58 p.m.	4-25 p.m.	224	„	„
„	July 17	2-25 p.m.	3-15 p.m.	48	„	„
„	Aug. 8	2-15 p.m.	3-50 p.m.	163	„	„
„	Sept. 12	2-15 p.m.	4-15 p.m.	4	„	„

Source	Date	Time of Collecting	Time of Investment	No. of Bacteria	B. Coli Comm.	B. Ent. Sporog.
Mixing Well	Oct. 9	2-10 p.m.	4-0 p.m.	37	Absent	Absent
"	Nov. 5	3-33 p.m.	5-20 p.m.	26	"	"
"	Dec. 4	2-7 p.m.	4-30 p.m.	29	"	"

Prescot—Rivington Water—Monthly Samples

Rivington	Jan. 23	4-45 p.m.	6-45 p m.	6	Absent	Absent
"	Feb. 20	3-50 p.m.	10-30 a.m.	5	"	"
"	Mar. 28	2-3 p.m.	5-0 p.m.	21	"	"
"	April 24	2-5 p.m.	10·50 p.m.	5	"	"
"	May 23	2-5 p.m.	4-0 p.m.	4	"	"
"	June 19	1-55 p.m.	4-25 p.m.	7	"	"
"	July 17	2-5 p.m.	3-15 p.m.	6	"	"
"	Aug. 8	2-5 p.m.	3-50 p.m.	8	"	"
"	Sept. 12	2-5 p.m.	4-0 p.m.	1	"	"
"	Oct. 9	2-5 p.m.	4-0 p.m.	27	"	"
"	Nov. 5	3-25 p.m.	5-20 p.m.	28	"	"
"	Dec. 4	2-5 p.m.	4-30 p.m.	28	"	"

Prescot—Vyrnwy Water—Monthly Samples

Vyrnwy	Jan. 23	4-40 p.m.	6-45 p.m.	8	Absent	Absent
"	Feb. 20	3-45 p.m.	10-30 p.m.	4	"	"
"	Mar. 28	2-0 p.m.	5-0 p.m.	16	"	"
"	April 24	2-0 p.m.	10-30 a.m.	11	"	"
"	May 23	2-10 p.m.	4-0 p.m.	3	"	"
"	June 19	1-50 p.m.	4-25 p.m.	12	"	"
"	July 17	2-15 p.m.	3-15 p.m.	21	"	"
"	Aug. 8	2-10 p.m.	3-50 p.m.	73	"	"
"	Sept. Sample not taken, reservoir being altered.					
"	Oct. 9	2-0 p.m.	4-0 p.m.	20	"	"
"	Nov. 5	3-30 p.m.	5-20 p.m.	16	"	"
"	Dec. 4	2-0 p.m.	4-30 p.m.	32	"	"

NOTE ON 'PINK-EYE' IN HORSES

NOTE ON 'PINK-EYE' IN HORSES

C. BALFOUR STEWART

AND

RUBERT BOYCE

During several weeks of the winter of 1900 an epidemic of 'Pink-eye' raged among the horses of Liverpool, assuming, at its height, so serious proportions that it was no unusual occurrence for 20 to 40 dead horses to have to be dealt with in one knacker's yard of the city.

The onset of the disease was very insidious, and the fatal termination often extremely rapid, for there were many cases in which the animals dropped down dead in harness. That the disease was of an infectious nature was evident from the fact of its running through a stable, and it was recognized as such in the treatment, for the affected animals were always isolated in those stables which were under proper veterinary charge.

Amongst those who had to do with horses there seemed to be a general opinion that the disease was the same as influenza in human beings, but, on enquiry, we were unable to meet with any instance of a case of influenza occurring from contact with sick horses, nor were we able to separate a microbe in any way similar to that of Pfeiffer.

An infectious disease of this nature, involving not only heavy loss on team owners but also considerable suffering to the animals, particularly those which were driven with the disease already on them, is one imperatively calling for investigation to discover the micro-organism concerned, and, if possible, to devise some means for conferring immunity.

Unfortunately for our investigation the epidemic came to an end somewhat suddenly, but we think it not uninteresting to put on record what few observations we made, with a view of prosecuting the enquiry further should an occasion again present itself.

Seven horses in all were examined, and the results of our observations are as follows :—

Horse 1. Suffering badly. A small quantity of blood was incubated in a sterile test tube, and another portion was inoculated on to the usual cultivation media, but no growth was obtained on any of the tubes, and the blood remained sterile.

Horse 2. Convalescent one week. The blood clotted very rapidly : it was likewise found to be sterile.

Horse 3. Convalescent. The blood also clotted rapidly, but did not form so large a buffy coat as in the previous case. It was sterile.

Horse 4. Suffering badly. The conjunctivae were greatly injected and a serous fluid was discharged from both eyes. The blood was tested and found to be sterile. A cover slip preparation was made from the eye discharge, it showed small bacilli and a tetra-coccus. Agar culture tubes and Petri dishes were inoculated with the eye discharge, and from this we obtained an almost pure culture of what, to save repetition, we called the characteristic bacillus, because of the uniformity with which it was met with in this and the following cases. It was a small diplococcus or diplo-bacillus which grew as a white opaque streak on gelatine, causing no liquefaction. On agar it grew more vigorously and slightly more opaque than B. coli. In glucose gelatine it formed gas. It showed motility in a hanging-drop preparation. The culture of one of the agar tubes was suspended in sterile water, and three guinea-pigs were inoculated with equal portions of the suspension. Two days afterwards one of the guinea-pigs had an oedematous swelling at the point of inoculation, it was killed, and there was found considerable subcutaneous oedema containing small diplococci. Agar tubes were inoculated from the oedematous fluid, and from the heart blood; those from the latter showed the characteristic bacillus both culturally and microscopically.

Horse 5. Suffering badly. Cultures from the conjunctiva showed the characteristic bacillus. A guinea-pig inoculated with some of the culture died in three days with an oedematous swelling similar to the last, and a cultivation from this gave the same bacillus.

Horse 6. Cultures were made from the lungs, trachea, nasal mucous membrane, conjunctivae, liver, spleen, kidney. The characteristic bacillus was recovered from the whole of the respiratory tract, but not from the organs. A guinea-pig inoculated with a culture from the trachea died in three days; the subcutaneous oedematous fluid showed the same characteristic bacillus, and cultivations were obtained from this and also from the blood. Guinea-pigs were also inoculated with cultures from the nasal mucous membrane, and from the conjunctivae with similar results. Other guinea-pigs were inoculated with the subcutaneous oedematous fluid and these died under similar conditions. Three guinea-pigs which had four days previously received an inoculation of 5 c.c. of blood serum from horse 1 were inoculated with a suspension of an agar plate culture of the nasal mucous membrane; three fresh guinea-pigs were also inoculated with a similar amount as controls. One of the controls died in two days under similar conditions as the above.

Horse 7. Suffering badly, cultures were made from the conjunctival discharge, and showed the characteristic bacillus. Afterwards, when the horse died, cultures were made from the nasal mucous membrane, bronchus of healthy lung, bronchus of pneumonic lung, and from the pneumonic lung substance. The same bacillus

was obtained from each specimen. The solid lung and its bronchus gave also a number of small colonies which proved to be a streptococcus, but it was without effect when inoculated into a guinea-pig.

Summary. In the above observations we found a small bacillus usually growing in pairs on artificial media, which from its reactions and cultures would appear to belong to the coli group. The whole respiratory tract of the affected animals seem to be infected, but from the fact that animals may die of the disease without showing 'pink-eye,' it is probable that the infection starts in the respiratory tract, and invades the conjunctiva through the nasal duct.

This bacillus is usually virulent to guinea-pigs, causing death in two to three days. From the fact that it was recovered constantly from the respiratory tract and conjunctiva of dead or diseased animals, there is strong presumptive evidence that it was the cause of the disease, but we have no experimental proof that it caused the typical disease under artificial inoculation.

The case would appear to be similar to those pathological conditions where the B. coli is found to have taken on an increased growth and virulence, as in some forms of enteritis.

REPORT OF THE LIBRARIAN

REPORT OF THE LIBRARIAN

The past year has been one of remarkable development for the Library. By the generosity of the Rev. S. A. Thompson Yates we have been able to acquire, from their commencement, the following valuable periodicals :—

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Beiträge z. Patholog. Anat. (Ziegler's Beiträge)
Centralblatt f. Bakteriologie. Pts. I and II
Centralblatt f. allg. Pathologie
Centralblatt f. Physiologie
Zeitschrift f. Hygiene, etc.
Zeitschrift f. Physiologische Chemie (Hoppe-Seyler)

together with several others noted below.

Moreover, by an arrangement with the Library Committee it was agreed to transfer certain periodicals from the Tate Library to the Departmental Library, on the condition that the previous numbers of those which were not complete should be purchased by the Department. In this way the Departmental Library has become fairly comprehensive in the literature of Bacteriology, Pathology, Physiology, Hygiene, and Neurology. There are still some lamentable gaps, but at present there are no funds by which they may be filled.

The number of exchanges has increased ; to several editors of old standing periodicals we owe thanks for their generous readiness to exchange.

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Chance Mountain, Montserrat (3,000 feet high), Cane Cultivation in the foreground

THE ISLAND OF MONTSERRAT

'And now on the leeward bow, another grey mountain island rose. This was Montserrat, which I should have gladly visited, as I had been invited to do; for little Montserrat is just now the scene of a very hopeful and important experiment. The Messrs. Sturge have established there a large plantation of limes, and a manufactory of Lime Juice, which promises to be able to supply, in good time, vast quantities of that most useful of all sea medicines, and I for one heartily bid God speed to the enterprise.'—

'At Last,' by the Rev. Chas. Kingsley.

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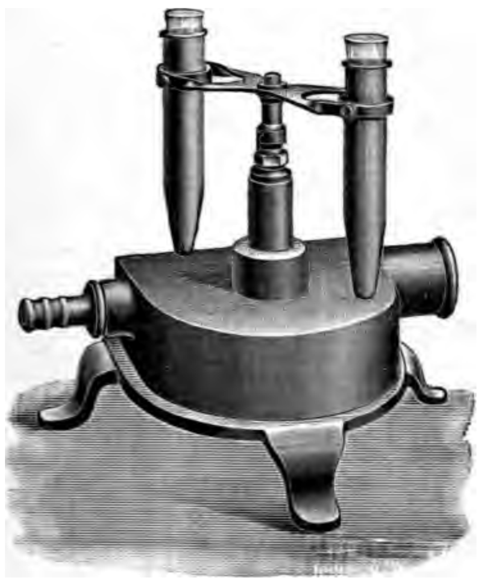

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